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GLYCOGEN IN PRODENIA ERIDANIA, WITH SPECIAL REFERENCE TO THE INGESTION OF GLUCOSE[1]

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INTRODUCTION

The importance of carbohydrates as a reserve food material for various animal organisms is well known. A reserve food supply is particularly important to insects such as the southern armyworm (*Prodenia eridania* (Cram.)) because of its relatively long period of pupation. Large numbers of the southern armyworm have been reared in the laboratories of the Division of Control Investigations for use in various physiological experiments. The insects have been reared in greenhouse cages and the larvae fed turnip and collard foliage, a diet high in carbohydrates. An investigation of some phases of carbohydrate metabolism in this insect has therefore been undertaken. The glycogen content has been determined at daily intervals throughout the life cycle, and the effect of the ingestion of glucose on the blood-glucose level and the tissue-glycogen content of sixth instars has been studied. The results of these experiments are reported in this paper following a review of the findings of other investigators with regard to the carbohydrate metabolism of various insects.

REVIEW OF LITERATURE ON CARBOHYDRATE METABOLISM IN INSECTS

OCCURRENCE OF GLYCOGEN

Glycogen was first demonstrated in insects by Bernard (*8, pp. 113–114*),[2] who described the larvae of "asticots" as "veritable sacks of glycogen." Bernard also found glycogen in the pupae of the same insects. Külz (*60*) found glycogen in the larvae, but not in the eggs, of *Calliphora vomitoria* (L.) (*Muscida vomitoria*).[3] Tichomiroff (*86*) showed that the yolks of hibernating eggs of silkworms (*Bombyx mori* L.) contained glycogen, but that during development the glycogen disappeared. Anderlini (*3*) found glycogen in all stages of the silkworm, the largest quantities occurring in the female pupae. In a preliminary report Bataillon and Couvreur (*6*), although giving no analytical data, found glycogen to be at a maximum the day after nymphosis, and then to decline rapidly until the amount remaining

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[2] Italic numbers in parentheses refer to Literature Cited, p. 525.

[3] Scientific names of insects in parentheses immediately following other scientific names are those appearing in the reference cited. Other scientific names are those currently in use according to the Division of Insect Identification of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

was too small to estimate accurately. Bataillon (*5*) later extended these observations and included his data. Dubois and Couvreur (*31*) found that glycogen reached a maximum of 53 mg. per six insects on the sixth day after climbing. The amount remaining at the end of the pupal period was insignificant.

Vaney and Maignon (*91*, *92*, *93*), reporting the daily changes that occur during the metamorphosis of the silkworm, found the greatest amount of glycogen (1.59 percent) just at the formation of the chrysalis, then a decline until emergence, and another rise after copulation. By histological methods they demonstrated glycogen in the cells of the fat body, in leucocytes, and in muscles, but not in the silk glands or the digestive epithelial or hypodermic cells. Eggs within the female moths contained glycogen. Tichomiroff's (*86*) finding that glycogen diminished during the development of the egg was confirmed. The eggs contained 2.85 percent of glycogen 2 to 4 days after oviposition and only 0.87 percent 7 to 8 days thereafter. In both pupae and adults the female contained considerably more glycogen than the male, the greatest difference being in the adults.

Bogojavlensky (*18*) found glycogen especially concentrated in the external lobes of the fat body of the silkworm, and present to a lesser extent in the internal lobes. Glycogen was also found in other organs, but Bogojavlensky concluded that this glycogen was slightly different from the fat-body glycogen since it seemed to have a different solubility. He found considerable glycogen in the midgut and in the terminal part of the rear gut. The test for glycogen in the silk glands was doubtful, although Bogojavlensky considered its presence probable. Glycogen depots were relatively abundant in the conjunctive tissue of the envelopes of the genital organs and were also present in the tracheae. Glycogen was always present in the walls of dorsal vessels and in pericardial cells, also in oenocytes and ganglia of the abdominal nervous system. It was often absent in muscle.

Using the roach *Blatta orientalis* L. (*Stylopyga orientalis*), Philiptschenko (*73*) found that glycogen was abundant in the cells of the larval fat body but was scarce in those of the adults. No seasonal variation in the amount of glycogen present in the tissues of the honeybee (*Apis mellifera* L.) was noted by Parhon (*70*), although she found a considerable seasonal variation in combustion and respiratory exchange.

Kotake and Sera (*58*) found a steady decrease in glycogen during the metamorphosis of the silkworm. As the exact age of their insects was not stated, they may have started their analyses too late to note the rise reported by other investigators.

According to Ronzoni and Bishop (*77*), carbohydrates found in the honeybee may be designated (1) as free sugar, determined as glucose, which is found in the blood and possibly to some extent in the cells; (2) as combined carbohydrate, part of which is a polysaccharide of lower molecular weight than glycogen and part of which is combined with protein in the blood; and (3) as glycogen. Glycogen is laid down exclusively in the cells but is released into the blood during the late stages of histological break-down.

The amount of carbohydrate present after acid hydrolysis was determined by Inouye (*50*) for various stages of the silkworm. Larvae, pupae, and adults contained 4.98, 4.37, and 5.71 percent, respec-

tively, calculated on a dry basis. Yamakawa (*97*) confirmed the results of other investigators in finding a decrease of glycogen during metamorphosis. Eggs of a single-brood strain of silkworms contained the largest amount of glycogen soon after they were laid, and the glycogen content then decreased steadily, according to Vaney and Conte (*90*). Straus (*82*) found that glycogen first appeared in the honeybee the third day in its larval life, and increased rapidly to a peak at pupation. At this time workers contained 32.48 percent of glycogen and drones 25.52 percent. The glycogen declined steadily until it had almost disappeared at emergence, but it rose again in the adult. According to Sturtevant (*83*), bees dead from foulbrood contained almost no glycogen. Pigorini (*75*) confirmed the results of previous workers as to the changes that occur in the glycogen content of the silkworm egg during development.

Ditman and Weiland (*28*) found that the metabolism of glycogen in pupae of the corn earworm, *Heliothis armigera* (Hbn.) (*Heleothis obsoleta* (*F.*)), was largely dependent on the temperature. At 86° F. values for the accumulation and decrease in the quantity of glycogen present were similar to those reported for other insects.

The development of *Gasterophilus intestinalis* (Deg.) (*Gastrophilus equi*) was studied over an 8-month period by Kemnitz (*56*, *57*), who found that between 31.1 and 14.1 percent of the dry weight was glycogen. He also found larvae of an unknown species of *Chironomus* to contain 16.67 percent of glycogen. Shinoda (*81*) found that pupae of the wild silk moth (*Dictyoploca japonica* (Moore)) contained only small quantities of glycogen, fat being its main reserve material.

The blowfly (unnamed species), according to Frew (*37*), did not contain glycogen or any other carbohydrate that yielded glucose on acid hydrolysis; both larvae and pupae were examined. Rudolfs (*79*) analyzed the tent caterpillar (*Malacosoma americana* (F.)) for glycogen throughout its life cycle. The glycogen fell from 2.79 percent at hatching to a few tenths of 1 percent when fully grown. In the same time the glycogen actually present increased from 27.9 mg. per 100 larvae to 375 mg. Rudolfs found glycogen in the muscles, leucocytes, fat glands, and eggs.

In *Culex pipiens* L., De Boissezon (*19*) found that glycogen was concentrated in certain fat cells, or trophocytes, of both larvae and imagoes. Because he did not find glycogen in the intestinal cells during digestion, he thinks that glycogenesis takes place in the fat cells.

Blanchard and Dinulescu, in an analysis of larvae (*15*) and pupae (*16*) of *Gasterophilus intestinalis*, *G. nasalis* (L.), and *G. haemorrhoidalis* (L.), found that glycogen rose steadily during the larval stage and fell off sharply during inanition. Most of their data were repeated by Dinulescu (*27*).

Evans (*33*) reported finding glycogen in the blowfly *Lucilia sericata* (Meig.), but from his description of the material he isolated this finding must be questioned. The material Evans isolated differed from glycogen in that it gave a reddish-brown color with iodine which did not return after boiling and no reduction was obtained after boiling with dilute acid. Total carbohydrate in the insect was obtained by hydrolyzing dried insect material with acid, clarifying, and determining reducing sugars by the Hagedorn-Jensen method.

Heller (*44*) found that the pupae of *Deilephila euphorbiae* (L.) contained 0.78 percent of glycogen. Timon-David and Gouzon (*89*) found that *Geoica utricularia* (Pass.) (*Pemphigus utricularius*) and *Pemphiga cornicularia* (Pass.) contained 2.007 percent of glycogen. Glycogen was not found in significant amounts in either the pupae or the prepupae of the wax moth (*Galleria mellonella* (L.)) by Crescitelli and Taylor (*24*). Beutler (*10*) did not find in the body of the honeybee any reserve carbohydrates from which the insect could replenish its blood sugar during starvation. Bialaszewicz (*12*) found that in the silkworm glycogen rose abruptly from 6 to 31.6 mg. on the sixth day after the fourth molt, dropped to 21 mg. during spinning, and rose again to 45 mg. in the pupal stage. Roy (*78*) could not detect glycogen in the body fluid of the wax moth. Babers (*4*) found 3.29 mg. of glycogen per 100 cc. of blood in the hemolymph of the sixth-instar southern armyworm. Paillot (*69*), using histological methods, found an increase in glycogen during the metamorphosis of the silkworm, the largest amounts being present in the fat tissue and epithelial cells of the digestive tube. He then stated that glycogen was not found in blood cells, pericardial cells, oenocytes, silk glands, Malpighian tubes, and probably not in muscle, but elsewhere in the same article he reported the presence of glycogen in oenocytes, pericardial cells, and occasionally in the Malpighian tubes. Kuwana (*61*) found 50 mg. of glycogen per 100 cc. of larval blood of the silkworm. During pupation glycogen rose to 130 mg. per 100 cc., the females consistently having more than the males.

SOURCE OF GLYCOGEN

Protein as a source of glycogen was suggested by Külz (*60*). He found that larvae of *Calliphora vomitoria* (*Muscida vomitoria*) that had been reared from glycogen-free eggs on egg white and horse meat contained glycogen, and he concluded that protein was the source of this glycogen. His conclusion does not necessarily hold true, however, since both egg white and horse meat contain carbohydrates. Although he did not mention glycogen by name, Abderhalden (*1*) found that the beetle *Anthrenus museorum* (L.) could synthesize all needed foodstuffs, both carbohydrates and fats, as well as chitin, from silk threads which were free from both fat and carbohydrate.

Couvreur (*23*) suggested that fat was the source of glycogen in the silkworm, since he obtained a rise in the glycogen content that corresponded to a fall in the fat content. Dubois and Couvreur (*31*) also considered fat to be a source of glycogen. Bataillon (*5*) considered the origin of glycogen to be histolytical, but Vaney and Maignon (*91*) did not think this probable, for they found glycogen in the largest quantities in cells that were most active during metamorphosis. Vaney and Maignon maintained that the fat-body cells resembled the liver cells of vertebrates in acting as centers for the elaboration of fat and glycogen. This view was also taken by Bogojavlensky (*18*), who found reserve glycogen mainly in the fat body of silkworms. He believed that it was formed locally from the monosaccharides obtained from the food. Weinland (*95*), from his work on *Calliphora*, did not consider the evidence presented by Couvreur sufficient to warrant the assertion that fat was the source of glycogen. Kotake and Sera (*58*) also disagreed with Couvreur; they found that both glycogen and fat decreased steadily during the chrysalis stage, but Yamakawa (*97*)

thought that in this stage carbohydrate was formed from fat and then utilized by the silkworm.

Kemnitz (*57*) presented evidence that glycogen in *Gasterophilus intestinalis* (*Gastrophilus equi*) was formed from the proteins in the stomach of the horse and later transformed into fat. The work of Kaneko (*52*) indicated that glycogen was built up in the gut epithelium of the silkworm from the carbohydrates in mulberry leaves. Bitō (*14*) found that silkworm pupae from larvae that had fed on leaves of *Cudrania triloba* contained less fat and glycogen than pupae from larvae fed on mulberry leaves, apparently because of the higher protein content of the *Cudrania*. Kato (*53*) found that, when partly mature mulberry leaves were fed to silkworm larvae, the digestion of soluble carbohydrates and the accumulation of glycogen in both larvae and pupae were greater than when older and younger leaves were fed. The addition of cane sugar in moderate amounts to the normal diet of the silkworm increased the weight of the body, cocoon, and silk. Kato, Miwa, and Negi (*54*) also found that the general health of the insects was improved, but no criterion of health was given. They found that 90 percent of the added sugar was digested, resulting in a remarkable increase in the fat and glycogen content of the insect. Kuwana (*61*) found that, after ingestion of glucose by silkworm larvae, the amounts of reducing substances in the blood following acid hydrolysis were greatly increased. He thought part of this was due to glycogen and perhaps part to a glucoprotein complex.

CHITIN

The metabolism of chitin has attracted the attention of only a few investigators. Weinland (*95*) considered that it was formed by the disintegration of protein. Bialaszewicz (*12*) found it to be at a maximum in the silkworm on the third day after the fourth molt. Paillot (*69*) thought the chitin of the silkworm cocoon was derived from glycogen. A rather complete analysis of the chitin in the various parts of *Periplaneta fuliginosa* (Serville) was made by Tauber (*84*).

REDUCING SUGARS

Glucose was not found in fly larvae, but was found constantly in adults, by Bernard (*8, pp. 113–114*). He concluded that glucose appeared during pupation. In a preliminary report Bataillon and Couvreur (*6*) stated that for a time the course of glucose in the silkworm pupae paralleled that of glycogen but the glucose continued to increase to a maximum while glycogen declined to a minimum just before emergence. Later Bataillon (*5*) reported that sugar did not appear in the silkworm until spinning was completed. His data are evidently those on which Bataillon and Couvreur based their conclusions. Maignon (*63*) agreed with Bernard in not finding glucose in the silkworm until after chrysalis formation, but stated that glucose was formed when tissues were incubated under sterile conditions under oil. His studies were extended by Vaney and Maignon (*91*), who found the appearance of glucose variable. They could not demonstrate glucose by the osazone test in the blood of silkworm larvae even when the gut was full of sugar. They consider that glucose was destroyed in the level of the epithelium of the intestine as soon as it reached the blood. Kawase, Suda, and Saitō (*55*) did not find reducing sugars in the blood of young silkworms.

Using the Hagedorn-Jensen method, Hemmingsen (*46*) determined the reducing substances in the blood of a number of insects, both before and after fermentation with yeast. He concluded that most of the reducing substances present were not glucose, since only a small portion was fermentable. He found that blood sugar rose after injection of insulin (*47*), and that insulin injected with sucrose did not hasten the disappearance of the sugar. In studies on the same insect by Wenig and Joachim (*96*), however, fermentable sugar disappeared within an hour after injection of insulin. The total reducing power of the blood did not change. Their normal value for the reducing power of the blood was 106 mg. per 100 cc., of which only 22.5 mg. was fermentable by yeast.

Medvedeva (*65*) concluded that insulin did not cause a specific reaction when injected into silkworms. Although in several experiments there was an increase in blood sugar after injection, he considered it due to a mechanical irritation set up by the process of injection rather than to the insulin. Adrenaline, on the other hand, caused a marked hyperglycemia. Blood sugar rose 71 percent above normal in the 15 minutes following injection and fell again almost as rapidly. Here, again, Medvedeva obtained a considerable hyperglycemia by the injection of water alone, and this effect also he attributed to a mechanical irritation by the syringe. He did not find a sex difference in glucose content in the hemolymph of normal imagoes.

Sturtevant (*83*) first found glucose in the honeybee on the third day of larval life. It increased rapidly during the feeding period but dropped off immediately after feeding ceased, and by the eighth day no reducing sugars were demonstrable. To the high concentration of sugar Sturtevant attributed the fact that *Bacillus larvae* White, the causative agent of American foulbrood, does not attack the normally feeding larvae, since the organism cannot develop in sugar solutions. Also using the honeybee, Bishop, Briggs, and Ronzoni (*13*) found 150 mg. of reducing substances per 100 cc. of blood. Beutler (*10*) found that only 2 percent of the reducing substances in adult bee blood was fermentable by yeast. After saccharose was fed the value rose. The rise was due to glucose, as in no case was fructose found. The blood sugar disappeared rapidly when it was not replenished. The queen bee had a high reducing value previous to and a low value subsequent to mating. Beutler (*11*) also found that the amount of sugar present was directly proportional to the energy output.

Blumenthal (*17*) described a micromethod for the blood analysis of insects and gave the average reducing value for the blood of *Romalea microptera* (Beauv.) (nymph) as 41.7 mg. per 100 cc., for *Melanoplus femur-rubrum* (Deg.) 35.3 mg., for *M. differentialis* (Thos.) 36.5 mg., for *Chortophaga virdifasciata* (Deg.) 34.0 mg., for *Encoptoloplus sordidus* (Burm.) 36.4 mg., and for *Popillia japonica* Newman 63.0 mg.

Frew (*37*) found a vigorous synthesis of glucose during the pupal period of the blowfly. Glucose was identified by its osazone. Frew suggested that the glucose originated from the histolysis of the various tissues. He was certain that glucose was not synthesized from fat either at the end of the larval period or early in the pupal period.

The blood sugar of *Deilephila euphorbiae* larvae was determined by Moklowska (*68*) to be 127 mg. per 100 cc. Approximately the same

value was given by Heller and Moklowska (*45*); they consider the amount of reducing substances present to be closely related to the stage of development.

Evans (*33*) found that in *Lucilia sericata* glucose fell during the first 13 days of metamorphosis and then remained constant during histogenesis. In *Tenebrio molitor* L. glucose declined from 144.2 mg. per 25 insects to 114.9 mg. during metamorphosis (*34*). Larvae starved for 8 days showed no carbohydrate change.

The reduction capacity of the hemolymph of several strains of silkworms was studied by Demjanowsky and Prokofjewa (*26*), who found that it rose sharply at each molt, fell sharply between molts, and reached a maximum at pupation. After emergence the hemolymph of the male moth showed a lower reduction capacity than that of the female. After oviposition, however, the reduction capacity of the female dropped sharply. After feeding the reduction capacity increased and reached a constant value in 5 hours. Ludwig (*62*) determined glucose in the Japanese beetle throughout the period of metamorphosis. In the late prepupae the glucose content was 0.50 percent, in young pupae 0.71 percent, in pupae 2 to 3 days old 0.62 percent, and at the end of the pupal period 0.84 percent.

In an attempt to find a point in the central nervous system of *Polysarcus denticauda* (Charp.) (*Orphania denticauda*) that controls glycemia, May (*64*) cauterized the cerebral and subesophageal ganglia and then determined the amount of reducing substances present in the blood. Although some difference between cauterized and normal insects was noted, May did not consider the difference significant. May also found that starving *Tettigonia viridissima* L. (*Locusta viridissima*) and *Carausius morosus* Brunn. (*Dixippus morosus*) for 24 hours markedly lowered the reducing value of their blood.

By isolating parts of the nervous sytem of fifth-instar silkworms Ermakov (*32*) was able to produce either hypoglycemia or hyperglycemia in the blood, depending on whether the anterior or the posterior portion of the insect was isolated. He obtained differences from normal as high as 59 percent and did not think them due to the quantity of food ingested. Crescitelli and Taylor (*24*) studied the changes in the reducing substances present in *Galleria mellonella* during pupation. Some glucose was present, since the osazone was isolated; but they did not consider that the entire reducing value was due to glucose. The largest amount present was 0.36 percent at pupation. No difference due to sex was observed. Yeager and Fay (*98*), studying the reducing value of the blood of roaches, *Periplaneta americana* (L.), that were killed under various conditions, found no significant difference between heat-killed insects, those fixed in acetic acid vapor, and those whose blood clotted under oil. There was no apparent diffusion from cell to plasma in the 15-minute clotting time, and the hemolymph from imagoes had about the same reducing power as that from large nymphs. The average value for the group of insects tested was 62 ± 14.4 mg. per 100 cc., calculated as glucose, although all the reducing power was not considered to be due to glucose.

Florkin (*35*) determined both total and fermentable reducing substances during the metamorphosis of the silkworm. The value for fermentable reducing substances was between 10 and 49 mg. per 100 cc. of blood and followed the glycogen content as determined by

Bataillon. Florkin (*36*) also determined the fermentable and nonfermentable reducing substances in the blood of *Hydrous piceus* (L.) (*Hydrophilus piceus*) and found that, while total reducing substances varied between 20 and 104 mg. per 100 cc., the fermentable portion was only 5 to 31 mg.

Roy (*78*) could not detect glucose in the blood of larvae of *Galleria mellonella*. Babers (*4*) found that in the sixth-instar southern armyworm the blood had a total reducing value of 65.9 mg. per 100 cc., of which only 11.1 mg. was fermentable by yeast. Becker (*7*) found that the larva of *Hylotrupes bajulus* (L.) did not increase in weight when glucose was added to its diet.

In a study of the reducing power of silkworm hemolymph, Kuwana (*61*) was not able to isolate glucosazone from the blood at any time. Using the Hagedorn-Jensen method, he found that a considerable part of the reducing power was due to a nonglucose fraction. When the sugar reagents were added to the protein-free blood filtrate and titrated immediately without the usual heating, a "cold value" was obtained. The total reducing value was obtained in the usual manner, and the total value minus the cold value gave the "hot value." Kuwana confirmed Demjanowsky and Prokofjewa's finding that the total reducing value of the silkworm blood increased sharply at each molt. The increase was due to the cold value, however, and not to glucose. The portion fermentable by yeast was small, not exceeding 20 mg. per 100 cc. Upon hydrolysis with sulfuric acid, the reducing substance greatly increased, and most of the increase was fermentable by yeast. Glucose given per os caused a rise in blood sugar that reached a maximum in 3 hours and then a sharp decline to a normal value in 6 hours. In the same time the reducing power of the hydrolyzate increased. Glycolysis did not occur in the silkworm blood.

UTILIZATION OF CARBOHYDRATES

Kemnitz (*57*), from an analysis of data obtained in his study of *Gasterophilus intestinalis*, calculated that under anoxybiosis 1.39 gm. of glycogen formed 0.41 gm. of carbon dioxide, 0.2 gm. of water, and 0.1 gm. of fat as fatty acids; they combine with glycerol to form neutral fat, which is stored as reserve material. The fermentable blood sugar in the insects studied by Hemmingsen (*46*) did not change during starvation. Beutler (*11*) found that the honeybee used sugar directly without first transforming it to fat.

Known carbohydrates were fed to bees by Phillips (*74*), and the availability was determined from the relative length of life of the bees. Haydak (*39*) reported that newly emerged bees could subsist for a long time on a pure sucrose diet. Bertholf (*9*) also fed bees on known sugars, and found that numerous carbohydrates could be digested but that glycogen could not be utilized. Bertholf (*9*) has summarized the work that has been done on the carbohydrate-digesting enzymes in insects.

In the tent caterpillar Rudolfs (*79*) found that when the muscles were active the glycogen content was reduced, but in the silkworm Bogojavlensky (*18*) found little or no glycogen in the muscle. He suggested that the muscles might not use glycogen as such or that his technique might not be able to detect the quantities present. It seems probable

that the latter surmise is the correct one. The work of Acqua (*2*) indicates that the silkworm itself cannot digest starch; however, the mulberry leaves contained ferments that accomplished the preliminary steps so that the silkworm used the starch as soluble sugar.

The respiratory quotient obtained by Johansson (*51*) for yellow mealworms (*Tenebrio molitor*) that fed on flour indicates the formation of fat from carbohydrate.

Rainey (*76*) concluded that in *Lucilia sericata* most of the fat was derived from protein. The carbohydrate content fell from approximately 10 percent at hatching to 7 percent in the prepupae.

Timon-David (*87*) suggested that certain wood-eating insects might synthesize fat from pentoses, xylose in particular. Later (*88*) he stated that, in general, insects that consume considerable amounts of polysaccharides are very rich in fats and that these fats apparently have their origin in the sugars.

That the honeybee can convert sugar and honey into wax was first reported by Huber (*49, v. 2, ch. 2*) and later confirmed by Dumas and Edwards (*29, 30*). Abderhalden (*1*) found that larvae of *Anthrenus museorum* followed a seemingly normal course when fed pure silk. Carbohydrates, including chitin, were synthesized from it. Brown (*21*) analyzed the excreta of *Automeris io* (F.) throughout the larval period, as well as the leaves on which it fed, and decided that only hexoses and disaccharides were utilized.

In *Galleria mellonella* Taylor and Steinbach (*85*) found an average respiratory quotient of 0.69 for the pupal period, which might appear indicative of an exclusive oxidation of fat. These authors do not consider the explanation so simple, however, since fat oxidation is usually accompanied by oxidation of carbohydrates. They suggest the possibility of the conversion of fat to glucose and the oxidation of this carbohydrate. Although no analytical data were given, Dinulescu (*27*) thinks glycogen was used during nymphosis of *Gasterophilus* for the formation of fat.

Heller (*40*) considers that in the silkworm metamorphosis is at the expense of carbohydrates, the protein being used for the formation of the cocoon; in *Deilephila* he found the metabolism during the corresponding period to be that of fat. Heller (*41*) found that 50 percent of the energy content of *D. euphorbiae* larvae remained in the adult, whereas only 36 percent was present in the silkworm. In starved *Deilephila* he (*42*) found that the metabolism of fat required 51.7 percent of the total energy exchange, of proteins 45.5 percent, and of carbohydrates only 2.8 percent. Heller (*43*) found that during pupation 42.2 percent of the energy requirement was supplied by carbohydrates, 44.8 percent by fat, and 13 percent by protein. In mature butterflies during a period of inanition 964 gram-calories were used up, of which 499 were supplied by fat, 435 by protein, and only 26 by carbohydrate.

In starved larvae of *Tenebrio molitor*, Mellanby (*66*) found that the glycogen reserve was used up in the first 8 days; during the remaining 20 days of starvation fat was the principal reserve used. From analyses of the larval fat of *Pachymerus bacteris* (L.) (*Pachymerus dactris*), Collin (*22*) decided that, although some of the fat was derived from the fat ingested, a considerable portion came from the carbohydrates present in the nut on which the insect fed.

Hiratsuka (*48*) found that the silkworm digested 40 percent of the carbohydrate in mulberry leaves. Brown (*20*) fed known substances to grasshoppers and analyzed their excreta. No monosaccharides or proteins were excreted, showing complete utilization. Ten percent of the glycogen fed was excreted. In the normal diet soluble sugars and proteins were utilized while the polysaccharides were, in the main, excreted unchanged. In adult Lepidoptera Kozhantshikov (*59*) found that glucose and saccharose were digested. Body weight of the females increased owing to the synthesis of fat for the formation of egg yolk. Kozhantshikov considers that the carbohydrate metabolism is directly connected with fertility and that Lepidoptera adults cannot use sugar directly.

Paillot (*69*) suggested that glycogen was used in forming the chitin of the silkworm cocoon and that during metamorphosis the cell nuclei actively utilized glycogen. Harnisch (*38*) found that under anaerobiosis the larvae of *Chironomus thummi* Kieffer used mostly mesenchymal glycogen. Fat-body glycogen seems not to have been used. Increases in glycogen were noted only after feeding. According to Kato, Miwa, and Negi (*54*) the addition of cane sugar to the mulberry-leaf diet of the silkworm was followed by an increased digestion of protein, fat, and ash. Walter and LaHue (*94*) found that corn earworm adults produced only nonfertile eggs when fed a fresh-honey diet, but when freshly fermented honey was fed the eggs were 93 percent virile.

EXPERIMENTAL MATERIAL AND METHODS

For the purpose of the experiments larvae of the southern armyworm that were reared in greenhouse cages on turnip leaves until the end of the third instar and then placed on collard foliage are called normal larvae. The larvae pupated in moist sand, the pupae were collected, and after emergence the adults were placed in oviposition cages and allowed to feed on a dilute cane-sugar solution.

Glycogen was determined by a slight modification of Pflüger's (*72*) method. After digestion with 30-percent potassium hydroxide, when entire insects were used, it was necessary to clarify the solution. This was done by adding 20-percent copper sulfate solution and 10-percent sodium tungstate solution to the neutralized solution. The precipitated "brei" was removed by centrifugation and washed with water. To the combined supernatants 95-percent ethyl alcohol was added to give a solution containing 66 percent of alcohol. After the solution had stood overnight, the precipitated glycogen was centrifuged, washed with 66-percent alcohol three times, and finally taken up in 2 cc. of hot water. The insoluble material was removed by quantitative filtration through a micro asbestos filter and washed until the volume of the filtrate was 5 cc. Sufficient concentrated hydrochloric acid was added to the filtrate to make the final concentration 2.2 percent with respect to hydrochloric acid, and the solution was hydrolyzed 3 hours in the water bath, cooled, carefully brought to pH 7, and diluted to 10 cc. or larger volume depending on the amount of reducing sugar expected from preliminary runs. The reducing sugar was then determined on 5-cc. aliquots by the micro Shaffer-Hartmann method (*80*). The glucose value was multiplied by 0.927

to obtain the amount of glycogen. For tissue glycogen the clarification step was not needed. Blood glycogen was determined on 1-cc. samples of pooled whole blood from several insects by the method of Creveld (*25*), except that the reducing sugars were determined by the micro Shaffer-Hartmann method. Blood sugars were run by the micro Shaffer-Hartmann-Somogyi technique as described by Peters and Van Slyke (*71*).

For the feeding experiments a paste was made by heating 2 gm. of glucose, 1 gm. of cornstarch, and 15 cc. of water just to the thickening point. Sandwiches were then made from disks of turnip leaves, cut out with a No. 10 cork borer, and portions of this paste containing approximately 12 mg. of glucose. One sandwich was offered to a single larva, and only those larvae that consumed an entire sandwich in the course of 30 minutes were used. After ingestion of a sandwich, the larva was placed on turnip foliage until used.

The term "tissue," unless otherwise designated, refers to the material remaining after removal of the blood and alimentary tract. Where blood analyses were made, the larvae were killed and their blood was partially fixed by immersing them for 1 minute in water at 60° C. The blood was collected after the snipping of a leg. After removal of the blood, the alimentary tract was dissected out and the remaining tissue dried in a weighed container at 80°. Control experiments showed no apparent loss of glycogen during the drying period. The dried tissues were weighed and analyzed individually for glycogen by the method mentioned above.

NORMAL VARIATION IN GLYCOGEN CONTENT

Beginning with eggs and ending with long-lived adults, analyses were made each day to determine the normal variation and the period of greatest glycogen accumulation. For the egg analyses several egg masses that were deposited within 2 hours of each other were used. The time of hatching was considered the beginning of larval life. The entire egg mass usually hatched within a short time after the first larvae hatched, and those larvae that hatched within 1 hour were grouped and reared together. Once a day the required number were removed, dried, and weighed, and the entire insects were analyzed for glycogen. The results of these analyses are shown in figure 1. Each point on the curve represents the average of the analyses of several groups of insects of the age indicated. That there is considerable difference in the glycogen content of male and female moths is clearly shown in figure 2.

The fat body has long been considered the storehouse of glycogen for the insect. This conclusion has been based almost entirely on histological evidence. Of the amount actually present, however, no record has been found. The fat body was therefore removed from mature sixth instars as completely as was conveniently possible with small forceps. The fat body from 10 larvae was combined, and when it was dry glycogen was determined in the usual manner. The weight of dry fat body obtained from 10 insects varied between 26.390 and 49.780 mg. and the percentage of glycogen between 17 and 27, with an average of 23.32 percent dry weight. The fat body is truly rich in glycogen.

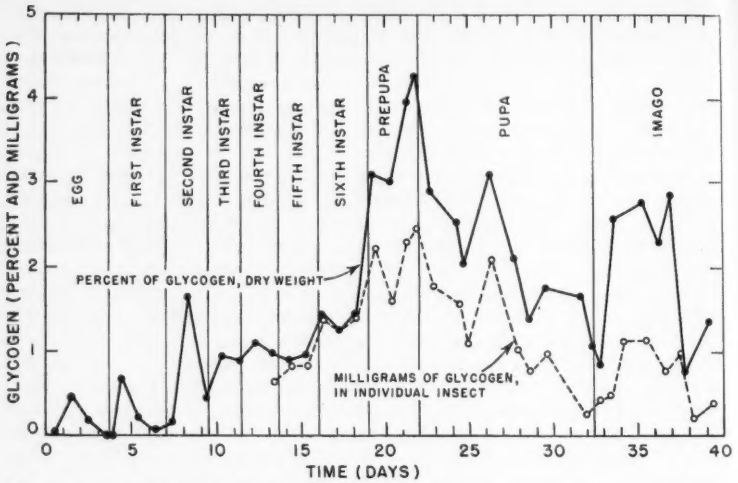

FIGURE 1.—Glycogen content of *Prodenia eridania* as indicated by daily analyses of entire insects throughout the life cycle.

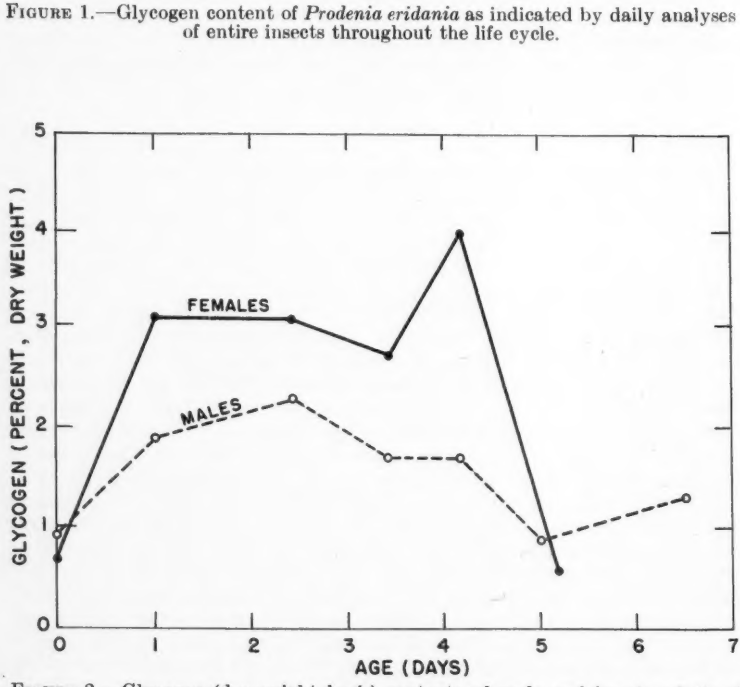

FIGURE 2.—Glycogen (dry weight basis) contents of male and female adults of *Prodenia eridania*.

EFFECTS OF GLUCOSE INGESTION

After the larvae had ingested glucose sandwiches, the hemolymph was analyzed for reducing substances at varying intervals beginning 15 minutes after normal feeding commenced. Analyses were made on the blood of individual insects, and each point on the curve in figure 3 represents the average reducing value, calculated as glucose, of approximately 25 insects.

When the blood from insects that had ingested a single sandwich was analyzed for glycogen, no definite variation from the normal glycogen content was found. In two instances a tremendous rise was obtained, the blood glycogen jumping to 69 and 178 mg. per 100 ml. of blood. Since in about 25 other tests no rise in glycogen content was found, it is believed that the high values obtained were due to

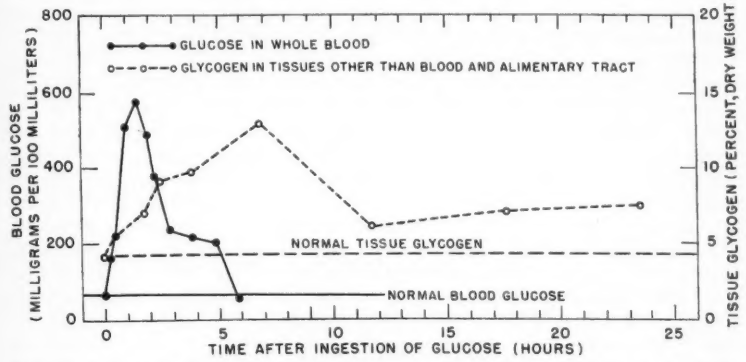

FIGURE 3.—Glucose and glycogen content of glucose-fed instars of *Prodenia eridania*.

some other cause, possibly incomplete digestion with potassium hydroxide. However, when larvae were allowed to feed overnight on turnip leaves smeared with glucose-starch paste, there was a definite increase in blood glycogen. The normal glycogen content of the blood of sixth-instar southern armyworms is between 1.3 and 4.5 mg. per 100 ml. The average of 10 analyses of 1-ml. samples was 3.29 mg. per 100 ml. Four samples of pooled blood from larvae that had fed on glucose overnight contained, respectively, 33.4, 17.42, 13.46, and 19.9 mg. of glycogen per 100 ml. The average reducing value of these samples was 133 mg., as glucose, per 100 ml. of blood, of which 63 mg. was fermentable by yeast.

In one experiment the cells and plasma were analyzed separately. The heat-fixed blood was centrifuged for 10 minutes at approximately 2,000 revolutions per minute, and the plasma was drawn off with a fine pipette. The cells were then washed twice with saline and the wash solutions combined with the plasma. In the cells 16.5 mg. of glycogen per 100 ml. was found and in the plasma 0.92 mg. It is probable that the small quantity found in the plasma was originally in the cells and was removed by the drastic treatment to which they

were subjected. After removal of the blood the alimentary tracts of these insects were dissected out, and glycogen was determined in the remaining tissue. The tissue from each insect was analyzed separately, and the results were averaged to obtain the point shown on the tissue-glycogen curve in figure 3. For the tissue of normal larvae with an average glycogen content of 4.26 percent, the standard deviation was 1.81. Four hours after the larvae had consumed a glucose sandwich the average tissue glycogen was 9.92 percent, with a standard deviation of 2.29. The standard deviation was not calculated for other points on the curve. The tissues from the larvae that had fed overnight on glucose contained 14.75 percent of glycogen, dry weight, and the tissue from individual larvae contained on an average 6.28 mg. of glycogen.

Hemolymph from normal sixth instars contained on an average 64.9 mg. of reducing substances, calculated as glucose, per 100 ml. of blood. Of this quantity only 11.1 mg. was fermentable by yeast, and was presumed to be glucose. Many investigators have reported similar low fermentable values in the insects tested, but many have also reported that in larval blood the glucosazone test was always negative. Upon examination under the microscope, however, glucosazone crystals were found in the blood of sixth-instar southern armyworms after treatment in the following manner. Three ml. of pooled blood from insects killed by immersion in water at 60° C. for 1 minute was diluted to 5 ml. with distilled water. Proteins were precipitated by the addition of 0.8 ml. of an acid cadmium sulfate solution (13 gm. of cadmium sulfate and 63.5 ml. of normal sulfuric acid per 100 ml.) and 1.1 ml. of normal sodium hydroxide (*67*). After dilution to 10 ml. and centrifugation, the supernatant was poured onto a mixture of 0.4 gm. of phenylhydrazine hydrochloride and 0.6 gm. of sodium acetate that had been finely ground together in a mortar. The test tube containing the mixture was protected from evaporation and placed in a boiling-water bath for 2 hours. The source of heat was then removed and the tube left in the bath until the latter had cooled to room temperature. Microscopic examination then showed the presence of widely scattered but definite clusters of glucosazone crystals.

Blood from insects that had ingested a glucose sandwich an hour before the blood was drawn also gave a positive glucosazone reaction. In this case, however, the glucosazone crystals were present in large numbers soon after the heating with phenylhydrazine was begun.

DISCUSSION

In general the curve showing the daily variation in the glycogen content of the southern armyworm is similar to the curves obtained by most other investigators for other insects. With the exception of Külz (*60*), who failed to find glycogen in the eggs of the fly *Calliphora vomitoria*, they seem generally agreed that the eggs of the various insects studied contain appreciable quantities of glycogen soon after deposition and that the glycogen then decreases steadily until minute quantities, if any, are found just before hatching. In the southern armyworm larvae glycogen appears soon after feeding commences, and increases to a peak at pupation. This also is in general agreement with other insects studied.

The time of greatest accumulation has been given as at pupation or just before or after. It is not surprising that a slight disagreement exists, if for no other reason than the extreme difficulty in defining the instant of pupation exactly. It is evident, however, that glycogen continues to be formed after the larva has ceased feeding. That this is an actual increase in the quantity of glycogen, and not a percentage increase due to loss of weight, is shown by the analyses of individual insects. Whether this glycogen is formed from soluble foodstuffs remaining in the alimentary tract before its complete evacuation or from autolyzing tissues is not clear. During the pupal period, as one would expect, there is a steady decline in the quantity of glycogen present. The chemical changes involved are not yet understood. The glycogen content rises again in the adult, and there is a distinct sex difference, the females having the highest glycogen content. Although little experimental evidence is presented to support the theory, it seems probable that the additional glycogen is the source of the energy required for egg production and of the glycogen found in the egg. It is not known whether the glycogen is deposited in the egg as such or whether the various cells synthesize it from other material. However, the evidence is overwhelming that glycogen plays a very important part in the general metabolism of insects.

At the time these experiments were started, very little quantitative experimental evidence was available that showed the type of foodstuff from which insects derive their glycogen supply. Most conclusions were based on the disappearance of one substance and a corresponding increase in another. After the completion of these experiments, however, the work of Kuwana (*61*) came to the writer's attention. This investigator, using the silkworm, has performed several experiments similar to those made by the present writer. The two curves for blood sugar after the feeding of glucose are very similar in shape. Kuwana obtained a peak value of 594 mg. of reducing substances as glucose in 3 hours, whereas the peak of 581 mg. obtained by the writer came in 1½ hours. The rapid rise and decline, however, were similar. In addition, Kuwana found a tremendous increase in reducing substances after acid hydrolysis of the blood of glucose-fed larvae, but he did not consider this to be due entirely to glycogen. The writer, of course, did not get an increase in blood glycogen after feeding only one sandwich. However, Kuwana apparently hydrolyzed whole blood with acid without the preliminary alkali treatment and alcohol precipitation used by the writer. It therefore seems probable that only a small part, if any, of the increase Kuwana found was due to glycogen. He did not follow the course of tissue glycogen after glucose ingestion. In the writer's experiments tissue glycogen increased definitely within a short time after glucose was fed, and the peak of glycogen formation came approximately 4½ hours after the blood-reducing value had reached its maximum. It is interesting to note that the rise in blood glycogen came only after long feeding on glucose-smeared leaves, as if it were an overflow into the blood after the tissues had reached a saturation point. The pH value of most insect blood is on the acid side. That of the southern armyworm is 6.53, and this acid condition may well retard glycogen formation and hasten its utilization in the blood.

The southern armyworm, therefore, possesses an efficient glycogen-synthesizing mechanism by which glucose can be readily transformed into glycogen. The site of this transformation is not known, but if one reasons by analogy with vertebrates, which of course is a risky procedure, the site of formation is also the site of the greatest glycogen concentration. If this is correct, then at least one function of the fat body is the same as that of the vertebrate liver.

The failure of other workers to identify glucose in larval blood by the osazone test has probably been due to their failure to use a large enough sample. Although the glucosazone test is very sensitive, in small samples the presence of glucosazone crystals may be easily overlooked, since at best there can be only a few crystals present when the sample contains only 0.05–0.2 mg. of glucose per milliliter.

SUMMARY

The glycogen content of the southern armyworm (*Prodenia eridania* (Cram.)) has been studied as part of an investigation of the carbohydrate metabolism of insects. A comprehensive summary of the literature on the carbohydrate metabolism of insects is included as a background for this study. This review shows that glycogen is widely distributed among insect species. Fats and proteins have both been suggested as the source of glycogen, but there is greater evidence that most of the glycogen comes from other carbohydrates. Glycogen and protein have been suggested as the source of chitin. Glucose has been found free in the hemolymph in only small quantities. The quantity of blood glucose seems to be related to the energy output. It is shown that insects are able to digest and utilize many carbohydrates.

In the experimental work with the southern armyworm the normal variation in glycogen content was first studied by analyzing entire insects at daily intervals throughout their life cycle. The effect of glucose ingestion was then studied by feeding sixth instars turnip-leaf sandwiches containing glucose and determining the blood glucose, blood glycogen, and tissue glycogen after varying intervals.

The results on normal variation were in general agreement with the findings of previous investigators on other insects. Glycogen was present in the egg, but disappeared at hatching, reappeared in the larvae after feeding had begun, and rose to a maximum at pupation; during pupation glycogen fell steadily and then rose again sharply in the adult. The female moths consistently contained more glycogen than the males.

Analyses of the fat body of mature sixth instars showed it to contain on an average 23.32 percent (dry weight) of glycogen.

In the feeding experiments the blood glucose rose sharply within 15 minutes, reached a maximum in 1½ hours, and returned to normal in 6 hours. Blood glycogen did not rise after one sandwich was fed. Tissue glycogen rose to a maximum in 7 hours after ingestion of a glucose sandwich.

Glucose was demonstrated in normal larval blood by the osazone test.

It is concluded that the southern armyworm possesses an efficient glycogen-forming mechanism by which glucose is transformed into glycogen. The chief site of glycogen formation appears to be the fat body.

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CALCIUM REQUIREMENTS OF GROWING PIGS[1]

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INTRODUCTION

The role of calcium and phosphorus in animal nutrition has received much attention in recent years, and many studies have been made to determine the need of these minerals in the nutrition of the pig. Much of the work has dealt with deficient rations and means of correcting the deficiencies. Recently the individual minimum requirements of these minerals have received the attention of investigators. Vitamin D in connection with these requirements has also been investigated (*3*, *6*, *9*).[3]

REVIEW OF LITERATURE

The requirements of phosphorus in the presence of vitamin D for the growing pig have been worked out by Aubel, Hughes, and Lienhardt (*1*), who place the minimum value between 0.27 and 0.30 percent when the calcium level is 0.8 percent. Spildo (*11*) and Axelsson (*2*) place the minimum value at 0.38 and 0.20 percent, respectively, and Mitchell and McClure (*10*) estimate it at 0.37 percent for a 30-pound pig and at 0.18 percent for a 200-pound pig. The lowest level fed by Dunlop (*6*) was 0.53 percent, at which level, with adequate calcium and vitamin D, he obtained satisfactory development. He does not report feeding a ration containing less than 0.53 percent phosphorus, and this, therefore, cannot be said to be a minimum level. Thus the minimum percentages of phosphorus required in the ration as so far determined are generally in fairly close agreement.

The requirement of calcium when vitamin D is supplied is reported by Dunlop (*6*) to be 0.34 percent, and he found a normal development of blood, bones, and body growth at this level whether or not vitamin D was supplied. Dunlop places considerable stress on the calcium-phosphorus ratio. He says, "A calcium level adequate at one ratio of calcium-phosphorus may be inadequate at another." In ascertaining his minimum calcium level Dunlop fed varying amounts of calcium with phosphorus levels at 0.53 percent or higher.

Other investigators have also reported the calcium requirements of pigs in the presence of vitamin D. Spildo (*11*) obtained satisfactory results with pigs which received 0.34 percent of calcium and 0.41 percent of phosphorus. Theiler and his associates (*12*) conducted experiments primarily on the effect of rations deficient in phosphorus or calcium or both. Their rations were deficient in calcium at a level of 0.10 percent and were adequate at a level of 2.0 percent. They did not feed any pigs a ration at a 0.30-percent level of phosphorus. Axelsson (*2*) estimates the percentage requirement of calcium at 0.37. His phosphorus level for this level of calcium was

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[3] Italic numbers in parentheses refer to Literature Cited, p. 542.

0.20 percent. Bohstedt (*5*) reports the most satisfactory results with levels of calcium at 0.32 and 0.41 percent in the presence of 0.28 and 0.29 percent of phosphorus. Aubel and his associates (*1*) produced satisfactory pigs with 0.8 percent of calcium and 0.3 percent of phosphorus. The calcium requirements in their study, however, were not directly under scrutiny. A level of 0.8 percent was fed so as to be sure that the pigs received ample calcium. Mitchell and McClure (*10*), on the basis of the composition of the body, estimate the requirements of calcium for a 30-pound pig to be 0.53 percent and state that it decreases gradually as the pig increases in weight, so that a 200-pound pig requires only 0.20 percent of calcium.

From these observations it would appear that with the exception of the work of Bohstedt (*5*), for which detailed data on blood and bone composition are not available, the minimum calcium requirements for growing pigs fed 0.3 percent of phosphorus, has not been determined. Aubel and his associates (*1*) found 0.3 percent of phosphorus to be the minimum level, a value which generally is approximated by the findings of other workers.

In view of the variations in the calcium requirements of pigs reported in the literature it seemed desirable to examine this question further. It is, therefore, the purpose of this paper to give information not only on the effects of a deficiency of calcium in the ration on the development of young pigs, but also to show the minimum requirements of calcium necessary to insure normal growth and bone and blood formation, when the phosphorus level is maintained at 0.3 percent and ample vitamin D is supplied.

EXPERIMENTAL PROCEDURE

Similar groups of pigs were fed rations which contained the same amounts of digestible nutrients, minerals, and vitamins, but different amounts of calcium. The response of the pigs, as reflected in body and bone growth, utilization of feed, and blood composition, was taken as the criterion for determining the amount of calcium necessary to meet the requirements for normal development.

The animals in each experiment were a uniform-appearing lot of purebred Hampshire pigs about 9 weeks of age and weighing about 40 pounds when the experiments were started shortly after weaning. Before being put on test the pigs were immunized against hog cholera and treated for roundworm. All pigs were housed in a well-lighted and well-ventilated building provided with individual concrete paved pens 6 by 8 feet in size. Each pen was provided with an outside concrete paved exercise area fenced with wire. The outside area allowed the pigs access to the direct rays of the sun and thus they were exposed to ultraviolet rays in considerable amount.

The animals were fed individually twice a day, the amount being determined by the normal-appearing pig with the smallest appetite. This arrangement insured all the pigs ingesting the same amount of food, so that differences in gains in weight and growth could not be attributed to variations in intake of food.

The basal ration consisted of 74 percent of pearl hominy, 10 percent of tapioca roots, 10 percent of blood meal, 4 percent of dehydrated alfalfa-leaf meal, 1.5 percent of dried brewer's yeast,[4] and 0.5 percent of iodized salt. Each pig received 5 cc. of cod-liver oil per

[4] This yeast was the Red Label brand of the Vitamin Food Company, Inc., New York City.

day. Enough monocalcium phosphate was used to raise the phosphorus content to 0.30 percent in all lots except lot 4, in experiment 2. In this lot, monosodium phosphate was used so that the calcium could be kept at 0.10 percent. Supplemental additions of calcium to vary the calcium level were made in the form of calcium carbonate. This ration was thought to contain, with the exception of the calcium, an adequate amount of all the elements known to be necessary for the normal growth of a pig.

The pigs were weighed every 28 days throughout the experiments. A determination of the calcium, inorganic phosphorus, and phosphatase in the blood was made at the beginning of each experiment and at the end of each 28-day period. A single sample of blood was drawn from the tail for these determinations. The Wang (*13*) method was used for the determination of calcium; phosphorus was determined by the Fiske and Subbarow (*7*) method, and phosphatase by the Bodansky (*4*) method.

The feeding period for each experiment was 24 weeks, at the end of which time the animals were killed and a pathological inspection of the carcasses was made. The femur and humerus bones of the right side of each pig were saved for chemical and physical analyses. A 75,000-pound Southwark-Emery hydraulic compression testing machine was used to determine the breaking strength of the bones.

EXPERIMENT 1

In experiment 1, conducted from December 2, 1938, to May 19, 1939, three lots of pigs were used. Lot 1 contained five pigs, and lots 2 and 3 four pigs each. The levels of calcium fed were 0.6 percent for lot 1; 0.8 percent for lot 2; and 1.0 percent for lot 3. The 1.0-percent level was included in order to observe the effect of an excess over the previously observed satisfactory level of 0.8 percent (*1*), and the 0.6-percent level was fed in an effort to locate a minimum level.

The growth, feed records, and composition of the blood and bone are shown in table 1, and the growth curves in figure 1.

TABLE 1.—*Effect of different calcium intakes, at higher levels, on the growth, the feed utilization, the blood composition, and bone formation in the pigs in experiment 1*

Lot No.	Pigs in lot	Calcium in ration	Phosphorus in ration	Average initial weight	Average final weight after 24 weeks' feeding	Average daily gain	Average daily feed	Feed required per 100 pounds of gain in weight
	Number	*Percent*	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	5	0.60	0.30	39.6	237.0	1.17	4.28	365.8
2	4	.80	.30	40.4	237.5	1.17	4.28	365.8
3	4	1.00	.30	41.6	236.7	1.16	4.28	368.9

Lot No.	Blood analysis at end of experiment			Average specific gravity of humeri	Average breaking strength of humeri	Bone analysis (dry fat-free basis)		
	Average calcium in 100 cc. of serum	Average inorganic phosphorus in 100 cc. of serum	Average phosphatase in serum			Average ash in femurs and humeri	Average calcium in femurs and humeri	Average inorganic phosphorus in femurs and humeri
	Milligrams	*Milligrams*	*Units*		*Pounds*	*Percent*	*Percent*	*Percent*
1	13.4	7.6	7.88	1.311	1,230	54.33	21.19	9.98
2	12.2	8.4	9.15	1.314	1,393	52.03	20.85	10.07
3	13.6	8.0	8.10	1.260	1,345	52.40	21.05	9.87

RESULTS

All the pigs in experiment 1 developed normally. It can be seen from the data in table 1, and from the curves in figure 1, that they were similar in all respects. They made almost the same daily gains, consumed the same amount of feed, had practically identical efficiency in utilizing their feed, had normal blood-serum calcium, phosphorus, and phosphatase content, and the bone formation was

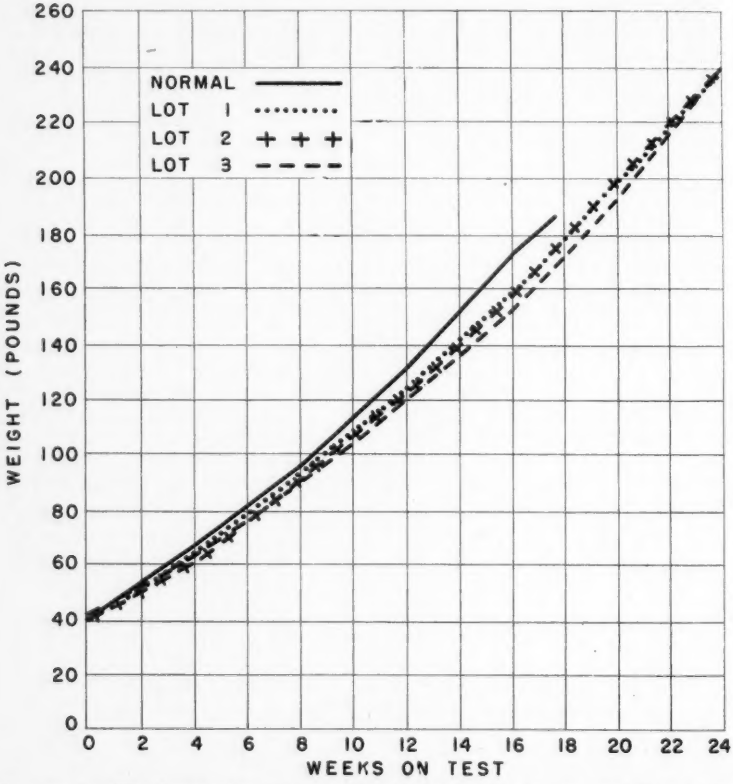

FIGURE 1.—Weights of pigs of lots 1, 2, and 3 with calcium intakes of 0.60, 0.80, and 1 percent, respectively, as compared with the weights of normal pigs. The curve for normal animals was drawn from data supplied by the Division of Animal Husbandry of the University of California and represents the average of the records of 457 pigs that had been on excellent rations (*8*).

normal as evidenced by the ash content and breaking strength of the bones. It is apparent from these data that a calcium level as high as 1.0 percent, and a level as low as 0.6 percent can be fed without abnormal results when ample vitamin D is supplied, and the phosphorus level is 0.3 percent of the ration. Under the conditions of this experiment, the requirements of calcium for the growing pig do not exceed 0.6 percent, the lowest level fed.

EXPERIMENT 2

The second experiment, conducted from December 6, 1939, to May 22, 1940, included four lots of pigs. Lot 4 contained three pigs, lot 5 five pigs, and lots 6 and 7 four pigs each.

In order that satisfactory comparisons might be made, experiment 2 was conducted with pigs of the same size and origin and in precisely the same manner as the first experiment. Lot 4 of experiment 2 received 0.10 percent of calcium; lot 5, 0.25 percent; lot 6, 0.41 percent; and lot 7, 0.55 percent. The high calcium level of 0.55 percent in this experiment was fixed just under the low level of lot 1 in the first experiment.

It was thought that the level of 0.55 percent in lot 7 would be near enough to the 0.6 percent of lot 1, experiment 1, to serve as a check on that level. In order to facilitate comparison, it was also planned in this experiment to control the intake of feed so that it would be similar to the amount ingested daily by the pigs in the first experiment.

The growth, feed records, and composition of the blood and bone of the second experiment are given in table 2 and the growth curves in figure 2.

TABLE 2.—*Effect of different calcium intakes, at lower levels, on the growth, the feed utilization, the blood composition, and bone formation in the pigs in experiment 2* [1]

Lot No.	Pigs in lot	Calcium in ration	Phosphorus in ration	Average initial weight	Average final weight after 24 weeks of feeding	Average daily gain	Average daily feed	Feed required per 100 pounds of gain in weight
	Number	*Percent*	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
4	3	0.10	0.31	41.5	182.3	0.83	3.79	456.6
5	[1] 5	.25	.30	40.8	219.0	1.06	4.23	399.0
6	4	.41	.30	41.4	232.5	1.13	4.30	380.5
7	4	.55	.30	41.9	232.7	1.13	4.27	377.8

Lot No.	Blood analysis at end of experiment			Average specific gravity of humeri	Average breaking strength of humeri	Bone analysis (dry fat-free basis)		
	Average calcium in 100 cc. of serum	Average inorganic phosphorus in 100 cc. of serum	Average phosphatase in serum			Average ash in femurs and humeri	Average calcium in femurs and humeri	Average inorganic phosphorus in femurs and humeri
	Milligrams	*Milligrams*	*Units*		*Pounds*	*Percent*	*Percent*	*Percent*
4	12.11	8.6	7.0	1.147	733	51.63	19.30	10.03
5	12.25	8.6	6.5	1.252	1,085	57.36	23.40	10.59
6	12.35	8.9	6.9	1.293	1,132	60.80	23.15	11.07
7	12.72	9.0	5.8	1.323	1,230	61.76	23.10	11.41

[1] Data for 1 pig in lot 5 not included.

RESULTS

All pigs in experiment 2 had smooth coats, good appetites, were thrifty, and made good gains throughout the experiment with the following exceptions:

In lot 4 (0.10 percent of calcium) all the pigs showed poor appetite. They developed severe diarrhea almost at the beginning of the ex-

periment and it lasted until the ninth week. During this time the pigs had poor appetites except on two or three occasions when their appetites returned and diarrhea ceased for short periods. They became coarse-haired and were nervous. After the tenth week, two of the pigs recovered to some extent and ate as much as the pigs in the other lots, so that by the end of the experiment they were fairly

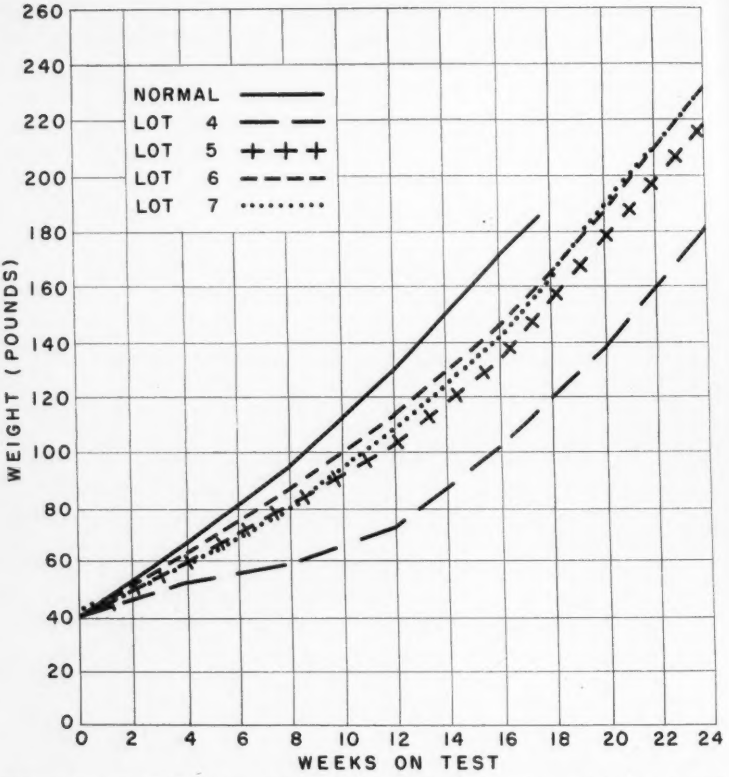

FIGURE 2.—Weights of pigs of lots 4, 5, 6, and 7 with calcium intakes of 0.10, 0.25, 0.41, and 0.55 percent, respectively, as compared with the weights of normal pigs. The curve for normal animals was drawn from data supplied by the Division of Animal Husbandry of the University of California and represents the average of the records of 457 pigs that had been on excellent rations (*8*).

smooth in coat, quite thrifty in appearance, and appeared normal except in weight. The third pig in this lot lost appetite the fifth week, developed severe diarrhea, and showed symptoms of weakness in the hindquarters. This pig did not recover its appetite until the twenty-first week. At slaughtering, the pigs in this lot had soft bones. It is significant that all the pigs on this low-level calcium ration exhibited the same calcium-deficiency symptoms described by Theiler and his associates (*12*). It is also significant that these pigs showed calcium-deficiency symptoms early and that after about 3

months of feeding they improved, especially in food consumption and thriftiness.

Two of the five pigs in lot 5, receiving 0.25 percent of calcium, gave indications of impaired appetite, unthriftiness, and diarrhea early in the experiment; pig No. 4 by the end of the third week, and pig No. 5, two weeks later. By the seventh week these pigs were obviously suffering from some deficiency. It was thought that possibly there might be a deficiency of water-soluble vitamin B in the ration. The yeast was accordingly increased to 5 percent in the ration of pig No. 4, and 5 mg. of nicotine acid was given orally each day to pig No. 5. These alterations in the feed were continued for 4 weeks, but there was no improvement in the condition of the pigs. By this time their appetites were very poor, they had developed severe diarrhea, and No. 4 was unsteady on his legs and slightly nervous (fig. 3, *A* and *B*).

By the end of 3 months pig No. 4 was paralyzed in the hindquarters and ate his food in a lying position. When it was apparent that he would not recover he was killed. An autopsy did not show any

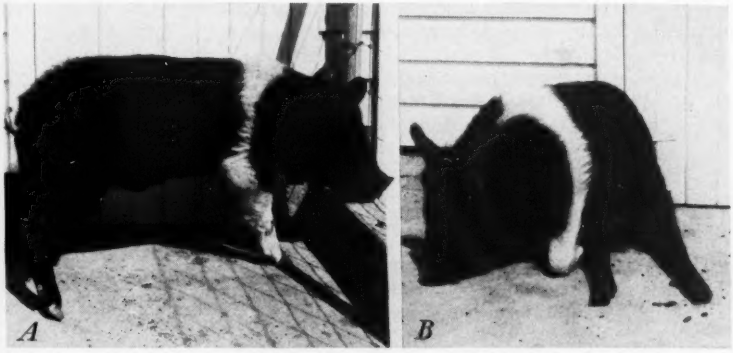

FIGURE 3.—Two views of pig No. 4, lot 5, experiment 2: *A*, After 8 weeks on ration containing 0.25 percent of calcium. Note the braced hind legs of this pig by means of which he keeps on his feet. *B*, After 12 weeks feeding, showing lying position and paralyzed hindquarters.

lesions that could account for the paralysis or the emaciated condition. The data in table 2 do not include those for pig No. 4.

Pig No. 5 showed much the same symptoms as pig No. 4, but never refused feed for more than 2 or 3 days at a time. From the tenth week until the end of the experiment this pig ate as much feed, most of the time, as the other pigs in the experiment. Although his appetite and appearance improved he never was as sleek-haired and thrifty as the other three pigs in this lot. Pigs Nos. 4 and 5 had soft bones when slaughtered.

Two of the five pigs in lot 5, which received 0.25 percent of calcium, showed calcium-deficiency symptoms early in the experiment, but one had recovered to a considerable extent by the end of the experiment. The other three seemed normal in appetite and growth, but at slaughter their bones were found to contain less ash and to break more easily than the bones of pigs receiving a higher level of calcium. It would seem, therefore, that the 0.25-percent level of calcium as fed in this lot might be close to the border line of calcium requirements for growing pigs.

Symptoms of calcium deficiency were not observed in lot 6 (0.41 percent of calcium) and in lot 7 (0.55 percent of calcium). The pigs in both lots developed and grew normally and had normal bones and blood as compared with lots 1, 2, and 3 of the first experiment.

DISCUSSION OF RESULTS

From the results obtained by feeding different levels of calcium it is evident that under the conditions prevailing in these experiments a level of 0.10 percent of calcium (lot 4, experiment 2) was inadequate for the normal development of pigs; they failed to gain normally, to utilize their feed efficiently, and to develop strong bones with normal ash content.

A 0.25-percent calcium level (lot 5, experiment 2) appeared to be near the border line or slightly below the calcium requirement. Two of the five pigs developed abnormally; the remaining three made normal gains in weight, but their bones contained a subnormal percentage of ash associated with a lowered breaking strength.

All pigs fed a ration containing 0.41 percent or more of calcium appeared to develop normally in every respect. Furthermore the feed consumption, utilization of feed, the composition of the blood, and the formation of the bone were similar with calcium levels ranging from 0.41 to 1.0 percent. These results indicate that raising the calcium level above 0.41 percent does not improve a feed for growing pigs.

Therefore, under the conditions of this experiment, when the level of phosphorus is 0.3 percent and adequate vitamin D is supplied, it would seem that a level of 0.25 percent of calcium is slightly below the minimum requirements while 0.41 percent is definitely adequate for the normal development of pigs.

EFFECT OF LOW-CALCIUM RATIONS ON THE BLOOD COMPOSITION

The effect of low-calcium rations on the composition of the blood has been reported by other investigators. Theiler and his associates (*12*) stated that the serum calcium was not significantly affected by the calcium of the ration. The findings herein reported are not entirely in agreement with this statement.

The data for the composition of the blood of the pigs in experiment 2 are shown in table 3. An inspection of these data shows that the serum calcium values of the lot 4 pigs and pigs Nos. 4 and 5 of lot 5, which showed calcium-deficiency symptoms, began to show a downward trend after 28 days on the low-calcium ration. This decrease in serum calcium continued for about 56 days, after which it increased, until at the end of the experiment the values were normal.

In the pigs that received an amount of calcium as low as 0.10 percent the blood calcium was not depressed below 8.92 mg. per 100 cc. of serum. It is not surprising, therefore, that low-calcium tetany was not observed in the pigs on a low-calcium diet in this experiment since such symptoms do not appear until blood calcium values fall below this value. It is worthy of note also that only the pigs showing slightly depressed serum calcium manifested any clinical evidence of calcium deficiency, such as loss of appetite, unthrifty appearance, and stiffness. These symptoms disappeared as the serum calcium returned to normal.

The reestablishment of serum calcium to normal after it had been slightly depressed would indicate that although a pig is receiving too little calcium in its diet, the system is able to maintain, apparently at the expense of bone calcium, almost a normal content of calcium in the blood. It should be emphasized, however, that ample vitamin D was provided. It seems surprising that pigs receiving a calcium-deficient diet sufficient to affect the appetite and produce the other clinical symptoms of calcium deficiency, are able to adjust themselves and subsequently recover their appetite and consume enough feed with its attendant amount of calcium to bring about satisfactory gains in body weight and a normal blood picture.

The data in table 3 also show that the inorganic phosphorus of the serum was little affected by the low-calcium rations.

TABLE 3.—*Calcium, inorganic phosphorus, and phosphatase content of 100 cc. of the blood serum from 5 pigs of lots 4 and 5, which showed calcium-deficiency symptoms, as compared with the means of these values for the pigs in lots 6 and 7, which received an adequate supply of calcium*

[Determinations made at 28-day intervals]

Lot No.	Pig No.	Initial			28 days			56 days			84 days		
		Calcium	Inorganic phosphorus	Phosphatase	Calcium	Inorganic phosphorus	Phosphatase	Calcium	Inorganic phosphorus	Phosphatase	Calcium	Inorganic phosphorus	Phosphatase
		Milligrams	*Milligrams*	*Units*	*Milligrams*	*Milligrams*	*Units*	*Milligrams*	*Milligrams*	*Units*	*Milligrams*	*Milligrams*	*Units*
Calcium deficient:													
4	1	12.40	8.0	3.6	13.36	9.0	3.4	10.52	8.1	6.1	8.92	8.0	5.4
	2	11.20	8.3	2.2	12.72	9.0	2.9	10.92	7.1	6.2	8.92	6.8	5.6
	3	11.66	8.0	2.8	13.14	8.9	2.5	11.24	7.0	5.0	9.63	6.2	2.1
5	4	12.02	7.0	3.3	12.92	8.8	2.8	10.86	7.7	7.3	11.56	9.5	2.3
	5	12.29	8.2	4.5	13.36	9.3	2.5	10.82	8.8	7.2	9.63	6.9	4.3
	6	12.40	8.2	1.4	13.56	9.8	3.8	12.24	10.2	9.8	11.31	8.8	5.8
	7	12.31	8.0	.9	13.56	9.5	3.6	14.82	10.0	11.3	11.55	7.9	6.7
	8	11.65	9.0	2.1	12.72	9.3	3.9	11.10	9.4	7.7	11.31	8.7	6.0
Calcium adequate:													
6	(2)	11.70	8.0	2.0	12.78	9.2	4.6	12.49	9.4	9.8	10.77	7.9	8.1
7	(2)	11.62	7.5	2.1	12.92	7.8	3.9	12.85	7.8	9.2	11.37	7.6	6.9

Lot No.	Pig No.	112 days			140 days			168 day		
		Calcium	Inorganic phosphorus	Phosphatase	Calcium	Inorganic phosphorus	Phosphatase	Calcium	Inorganic phosphorus	Phosphatase
		Milligrams	*Milligrams*	*Units*	*Milligrams*	*Milligrams*	*Units*	*Milligrams*	*Milligrams*	*Units*
Calcium deficient:										
4	1	10.27	9.6	10.1	10.47	8.1	7.9	11.90	8.9	6.1
	2	11.13	8.8	9.6	10.41	8.0	11.7	12.26	9.0	6.1
	3	10.70	7.0	6.7	9.86	7.1	11.2	12.17	7.9	8.9
5	4	11.23	8.5	11.2	11.40	7.9	7.8	(1)		
	5	10.91	8.1	8.5	11.49	8.2	10.9	12.30	9.3	7.3
	6	11.02	8.7	7.0	11.23	8.3	8.7	12.00	8.4	6.6
	7	11.88	9.1	9.5	11.56	8.2	10.3	12.40	8.6	6.0
	8	11.56	8.6	10.0	10.82	8.0	9.8	12.30	8.2	6.4
Calcium adequate:										
6	(2)	11.42	8.3	9.9	11.23	7.9	10.5	12.35	8.9	6.9
7	(2)	12.36	8.7	8.9	11.24	8.3	6.9	12.72	9.0	5.8

[1] Killed for autopsy.

[2] Mean of the lot.

After the pigs had been on experiment 84 days and the blood calcium had dropped to its lowest level, the serum phosphatase was

also somewhat below the level of that in the serum of the pigs receiving an adequate amount of calcium. The average of the five calcium-deficient pigs was 3.9 units per 100 cc. of serum, while that of lots 6 and 7 receiving an adequate amount of calcium and showing no calcium-deficient symptoms was 7.5 units.

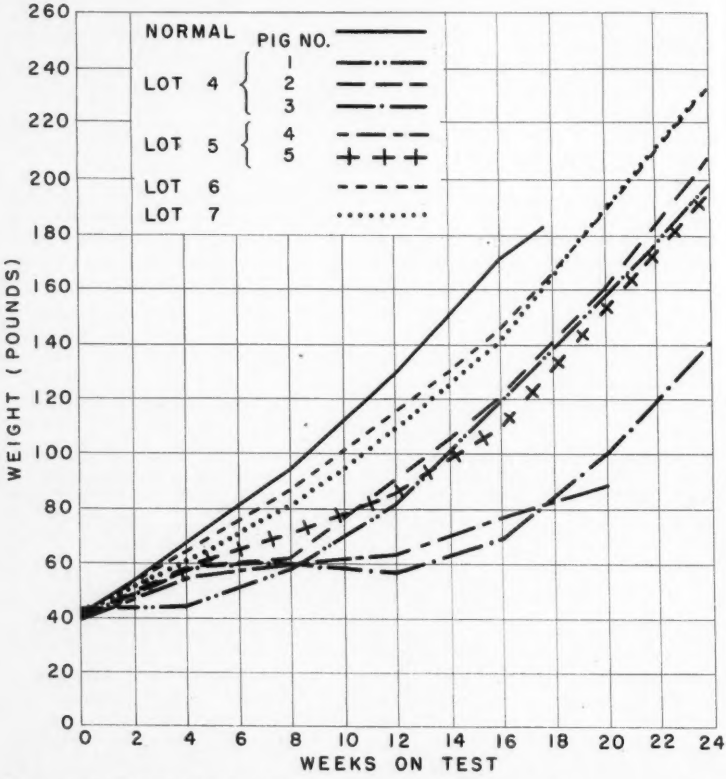

FIGURE 4.—Weights of the three pigs in lot 4, and pigs Nos. 4 and 5 of lot 5, showing calcium-deficiency symptoms, as compared with the weights of normal pigs, and with those of lots 6 and 7 which received adequate calcium. The calcium intakes of the animals in lots 4, 5, 6, and 7 were 0.10, 0.25, 0.41, and 0.55 percent, respectively. The curve for normal animals was drawn from data supplied by the Division of Animal Husbandry of the University of California and represents the average of the records of 457 pigs that had excellent rations (*8*).

EFFECT OF LOW-CALCIUM RATIONS ON DAILY GAINS AND BODY WEIGHT

One of the most interesting results of the feeding of low-calcium rations to young pigs was the effect produced on daily gain and body growth. The data for these two factors are recorded in table 4 and shown graphically in figure 4.

TABLE 4.—*Daily gains and body weights of 5 pigs from lots 4 and 5, showing calcium-deficiency symptoms during experiment 2, and the means of these values for the pigs in lots 6 and 7, which received an adequate amount of calcium*

[Weights recorded at 28-day intervals]

Lot No.	Pig No.	Initial body weight	28 days		56 days		84 days		112 days		140 days		168 days	
			Daily gain	Body weight	Daily gain	Body weight	Daily gain	Body weight	Daily gain	Body weight	Daily gain	Body weight	Daily gain	Body weight
Calcium deficient:		*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
4	1	43.3	0.02	44.0	0.46	57.0	0.85	81.0	1.35	119.0	1.35	157.0	1.46	198.0
	2	38.6	.65	57.0	.17	62.0	.96	89.0	1.10	120.0	1.42	160.0	1.71	208.0
	3	42.6	.55	58.0	.07	60.0	−.15	56.0	.42	68.0	1.14	100.0	1.46	141.0
5	4	38.6	.55	54.0	.21	60.0	.10	63.0	.50	77.0	.39	88.0	(1)	
	5	41.6	.58	58.0	.42	70.0	.57	86.0	.85	110.0	1.53	153.0	1.50	195.0
Calcium adequate:														
6	(2)	41.4	.76	62.7	.85	86.7	.99	114.5	1.12	146.0	1.48	187.7	1.60	232.5
7	(2)	41.9	.62	59.5	.76	81.0	1.00	109.2	1.16	141.7	1.67	188.5	1.57	232.7

[1] Killed for autopsy. [2] Mean of lot.

The pigs in lot 4, and pigs Nos. 4 and 5, lot 5, which showed calcium-deficiency symptoms, made slow daily gains and poor body growth almost from the beginning of the experiment until the one hundred and twelfth day. After this period of slow growth daily gains materially improved so that during the latter part of the experiment their gains were almost as good as those of the pigs in lots 6 and 7, which received an adequate amount of calcium (table 4). The initial rate of gain for calcium-deficient pigs was quite different from that of normal pigs or that of the pigs in lots 6 and 7 which received an adequate amount of calcium, as is shown in figure 4.

The recovery in the rapidity of daily gain seems to have preceded the depression and recovery of the serum calcium values discussed previously. Thus it appears that pigs as they increase in age are able to recover from the effects of a limited calcium deficiency even though the level of calcium in the ration is unchanged. This would indicate that the requirements for calcium are greater in early life and become less as the pig matures. These findings support those of Mitchell and McClure (*10*) in respect to the calcium requirements for pigs.

SUMMARY

The results of an investigation to determine the effects of feeding calcium at different levels in the rations of 28 young pigs are reported.

Two experiments, one with three lots of pigs and one with four, were carried on for 24 weeks.

Data are presented to show (1) the effect of a deficiency of calcium in the ration on the growth and development of pigs, and (2) the minimum amount of calcium necessary in the ration for normal growth and development.

The results obtained indicate that the abnormalities resulting from feeding low-calcium rations are: (1) Anorexia; (2) an unthrifty appearance, characterized by emaciation and a rough coat of hair; (3) usually, but not always, unsteadiness in legs and finally posterior

paralysis; (4) a failure of normal growth and development of bone; and (5) poor utilization of feed.

Under the conditions of the experiments a level of 0.25 percent of calcium in a ration was found to be inadequate, whereas 0.41 percent was definitely adequate for the normal development of young pigs.

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DROUGHT TOLERANCE IN SNAP BEANS[1]

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INTRODUCTION

When the U. S. No. 1 Refugee bean was first tested at various places in the United States in 1934 it was reported to possess some tolerance to drought conditions. One report indicated that it had made a fair yield of snap beans while standard bean varieties in the same test did not produce a crop. Because of these reports, attempts were made to determine the degree to which U. S. No. 1 Refugee possessed the ability to yield well under adverse conditions of soil moisture, and whether strains related to it possessed more or less tolerance to the same conditions. The tests were conducted at the United States Horticultural Field Station at Cheyenne, Wyo.

MATERIAL AND METHODS

The U. S. No. 1 Refugee bean was selected from the tenth generation of a cross of Stringless Green Refugee × Wells Red Kidney. In developing this variety several other slightly different strains were carried along but not chosen for introduction. Some of these strains were used in this test. U. S. No. 1 Refugee is closely related to U. S. No. 5 Refugee, since the parents of the latter variety are U. S. No. 1 Refugee and a mosaic-resistant Refugee rogue (a strain of Corbett Refugee). U. S. No. 5 Refugee was released in 1935. All the strains with dashes separating parts of the numbers are from the F_3 or later generations of U. S. No. 1 Refugee × Refugee rogue and are essentially variable sister lines of U. S. No. 5 Refugee. It should be pointed out that these F_3 lines were not fixed, and as they were increased there was undoubtedly differential survival of different types within the genetic complex. All other numbered strains are closely related to U. S. No. 1 Refugee. Five commercial varieties widely grown in the United States were carried for 4 years as controls, and in addition Burpee Stringless Greenpod, for 3 years.

In all years the varieties were planted in blocks planned for variance analysis. However, treatments and number of replications varied from year to year, as did somewhat the size of plot. The rows were 3½ feet apart, 50 feet long in 1935, and 25 feet long in the other 3 years. There were four plots of each strain in 1935, six in 1936, and eight in the last 2 years. In 1935 all plots were irrigated lightly once during the season. In 1936 and 1937 no irrigation was applied. In 1938 normally irrigated plots were randomized in with the unirrigated plots, to measure better the effect of drought conditions.

[1] Received for publication January 2, 1941.

All data are based on the accumulated yields per plot of snap beans (*Phaseolus vulgaris* L.) made on seven or eight picking dates of each season. Records were kept of the number of pods per pound at each picking.

EFFECT OF DROUGHT ON YIELD

The yields of snap beans by varieties and strains, grown with and without irrigation, are shown in table 1.

TABLE 1.—*Yield of snap beans by 35 varieties and strains in tests at Cheyenne, Wyo., 1935–38*

Varieties and strains	Acre yield						
	1935 (1 light irrigation)	1936 (no irrigation)	1937 (no irrigation)	1938		Average for 3 years 1935–37	Average for 4 years 1935–38
				No irrigation	Normal irrigation		
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
367	12,250	4,361	4,740			7,117	
344	11,347	4,208	4,865			6,807	
388–6–2	11,324	4,464	4,398	5,140	7,529	6,729	6,630
286	11,203	4,464	5,285	4,916	6,260	7,014	6,658
285	11,044	4,184	5,170			6,799	
282	11,001	3,837	4,849			6,562	
229	10,779	4,291	5,026			6,699	
305	10,748	4,615	4,906	4,094	3,933	6,756	6,071
391–15–2	10,659	3,694	3,907	4,430	4,082	6,087	5,629
388–1–5	10,612	3,287	3,975	4,431	8,139	5,958	6,040
287	10,534	4,070	5,213	3,871	5,177	6,606	6,085
388–8–5	10,351	3,663	4,029			6,014	
304	10,332	4,527	5,221	4,343	5,152	6,693	6,207
280	10,316	3,918	5,003			6,412	
919	10,215	4,993	5,116	4,692	4,779	6,775	6,265
283	10,149	5,159	5,433			6,914	
309	10,071	4,337	5,048	4,493	4,742	6,485	6,018
U. S. No. 5 Refugee	9,916	3,077	4,031	6,472	7,355	5,675	5,984
391–1–4	9,943	4,952	5,485	4,916	6,596	6,793	6,534
228	9,818	4,410	4,838	4,244	7,156	6,364	6,198
308	9,706	4,889	4,906			6,500	
391–15–11	9,682	3,881	4,398			5,987	
391–15–4	9,593	3,272	3,237			5,367	
388–7–6	9,527	3,770	4,157			5,818	
U. S. No. 1 Refugee	9,223	3,993	4,927	4,629	5,015	6,048	5,741
328	9,157	3,261	3,784			5,401	
Stringless Green Refugee [1]	9,106	4,343	3,959	5,053	7,940	5,803	5,976
Giant Stringless Greenpod [1]	8,846	4,672	4,138	4,095	4,057	5,885	5,433
342	8,768	3,942	4,540			5,750	
284	8,745	4,304	5,112	5,090	5,849	6,054	5,908
Full Measure [1]	8,589	3,612	3,343	4,406	3,783	5,181	4,910
341	8,367	3,230	4,455			5,351	
Brittle Wax [1]	7,442	3,430	4,068	3,273	3,535	4,980	4,586
New Stringless Greenpod [1]	6,462	4,029	3,864	2,639	3,534	4,785	4,360
Burpee Stringless Greenpod [1]	5,948	4,571	4,122			4,880	
Total	341,863	143,710	159,548	85,227	104,613		
Average	9,768	4,106	4,559	4,486	5,506		

[1] Commercial variety.

In 1935 the yields for an average of four plots varied from 12,250 pounds per acre for No. 367 (a U. S. No. 1 Refugee sib) to 5,948 pounds for Burpee Stringless Greenpod. A difference of 2,158 pounds was required for significance between strain means if odds of 19 to 1 are accepted as a criterion (table 1). In 1936 the yields for an average of six plots varied from 5,159 pounds for No. 283 (a U. S. No. 1 Refugee sib) to 3,077 pounds for U. S. No. 5 Refugee. A difference of 1,301 pounds was required for significance between the means.

In 1937 the yields varied from 5,485 pounds for No. 391–1–4 to 3,237 pounds for No. 391–15–4, with a difference of 412 pounds being required for significance.

In 1937 the plantings were so made that there were two series of plots to a block, and four blocks. The first of these blocks was approximately 40 feet from a windbreak to the west of the planting. Figure 1 shows the plots with block 1 in the foreground. The windbreak, consisting of a single row of poplars, is just out of the picture at the right. It was found in 1936 that the plots nearest the windbreak made somewhat greater yields than those farther away. The method of planting adopted in 1937 made it possible to measure this effect of the windbreak. Thus, large variances were obtained for both blocks and series (*F* values exceeding the 1-percent point), and the average yield decreased as the distance from the windbreak increased. However, the relative ranking of the varieties and strains remained approximately the same regardless of proximity to the windbreak.

FIGURE 1.—View of the field plots in 1937, with block 1 in the foreground. The western windbreak is just out of the picture at the right.

The average yield for the 35 varieties and strains in the block nearest the windbreak was 5,080 pounds, while the average for these same varieties and strains in the fourth block was 3,859 pounds. The averages for the second and third blocks were 4,758 and 4,526, respectively. Since only 135 pounds is required for significance in this case, all the differences between blocks are significant.

In 1938, seed of many of the strains was exhausted; so only 19 varieties and strains instead of 35 were carried. A comparison of the average yields of these 19 varieties and strains for the 4 years shows

that No. 286, a strain closely related to U. S. No. 1 Refugee, ranked first, and No. 388–6–2, a strain closely related to U. S. No. 5 Refugee, was second. Of the commercial varieties, Stringless Green Refugee ranked twelfth, Giant Stringless Greenpod sixteenth, Full Measure seventeenth, Brittle Wax eighteenth, and New Stringless Greenpod nineteenth. U. S. No. 5 Refugee ranked eleventh and U. S. No. 1 Refugee fourteenth.

If all 35 varieties and strains are included and ranked according to yield for the average of all plantings, it is found that strains 367, 283, 344, and 285 yielded significantly more than Stringless Green Refugee, the highest yielding commercial variety, which ranked twenty-second with a yield of 5,976 pounds. The other commercial varieties ranked as follows: Giant Stringless Greenpod, twenty-eighth; Full Measure, thirty-second; Burpee Stringless Greenpod, thirty-third; Brittle Wax, thirty-fourth; and New Stringless Greenpod, thirty-fifth; with acre yields of 5,433, 4,910, 4,880, 4,586, and 4,360 pounds, respectively. A significant difference for the comparison of mean yields for 4 years is 746 pounds, while if the 4-year averages are compared with the averages of the first 3 years a difference of 800 pounds is required.

No method of ranking based on either irrigated or nonirrigated plots or both will give a very high ranking to the commercial varieties. With the exception of Stringless Green Refugee, the commercial varieties are always near the bottom.

The results of irrigation in 1938 were complicated by the appearance of bacterial blight (*Bacterium phaseoli* E. F. Smith and *Bact. medicaginis* var. *phaseolicola* Burk.).[2] An effort was made to evaluate the influence of bacterial blight by estimating the amount of damage done on a scale of 0 to 5,[3] and at the end of the season the number of plants dead from blight was recorded. Adjustments on the basis of dead plants gave unreasonably high yields for the remaining plants, so further consideration was not given to adjusted yields. A variance analysis of the damage ratings was made in which the *F* values for irrigation and for varieties both exceeded the 1-percent point. Significant differences in disease evaluation between the mean of 80 irrigated and 80 nonirrigated plots was calculated to be 0.162. The actual mean difference is 0.344. The mean value of the irrigated plots was 3.888, while that for nonirrigated plots was 3.544.

Comparisons of blight damage within and between varieties are, however, more difficult, since the seed for most of the commercial varieties had been grown in relatively blight-free areas, while the seed of U. S. No. 1 Refugee and U. S. No. 5 Refugee and all other numbered strains had been grown at Greeley, Colo., where bacterial blight is fairly common every year. Four varieties showed a nonsignificant decrease in bacterial blight with irrigation, 12 varieties showed a nonsignificant increase, and only 4 showed a significant increase. In the nonirrigated beans, U. S. No. 5 Refugee and Full Measure both had the same low average value for bacterial blight (2.625 mean of 4 plots), and Stringless Green Refugee was not significantly different.

[2] Synonyms: *Phytomonas phaseoli* (E. F. Smith) Bergey et al., and *P. medicaginis* var. *phaseolica* Burkholder.

[3] 0=healthy; 5— =very severe infection.

All other strains and varieties had significantly more blight damage than did the first two varieties. In the irrigated plots, U. S. No. 5 Refugee was low, with an average value of 3.125, while 10 strains showed significantly greater injury.

EFFECT OF DROUGHT ON SIZE OF POD

To make possible a study of the effects of drought on size of pod, counts were made at each harvest date of the number of pods per pound for each variety and strain.

The variations in number of pods per pound at the various harvest dates are shown graphically in figures 2, 3, 4, and 5 so that comparisons

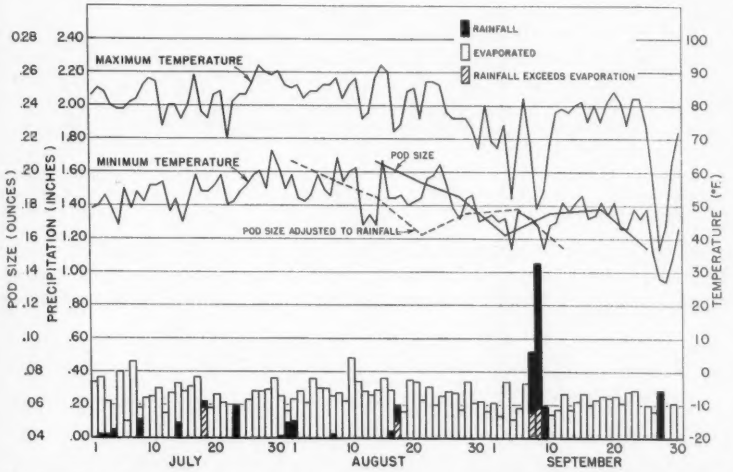

FIGURE 2.—Comparison of effects of maximum and minimum temperatures, evaporation, and precipitation on average pod size of 35 varieties and strains of snap beans, 1935.

may be made between them and such factors as precipitation, maximum and minimum temperatures, and evaporation. The average pod size in ounces is shown by a solid line, whereas a broken line represents pod size for dates 14 days prior to the actual dates of harvest. This adjusted line was set forward by a period of 14 days because an examination of the data showed that as a rule this was the length of time that usually elapsed between the occurrence of rainfall and response by the plants as measured by an increase in the size of the pods. In those instances where this rule holds true, the maximum and minimum points of the adjusted line should coincide with periods of rainfall and drought, respectively. In a few instances there appears to have been a period of only 6 or 7 days between rainfall and an increase in pod size. A more exact measurement of the time required for the plants to respond is not possible, since the harvests were made only at weekly intervals.

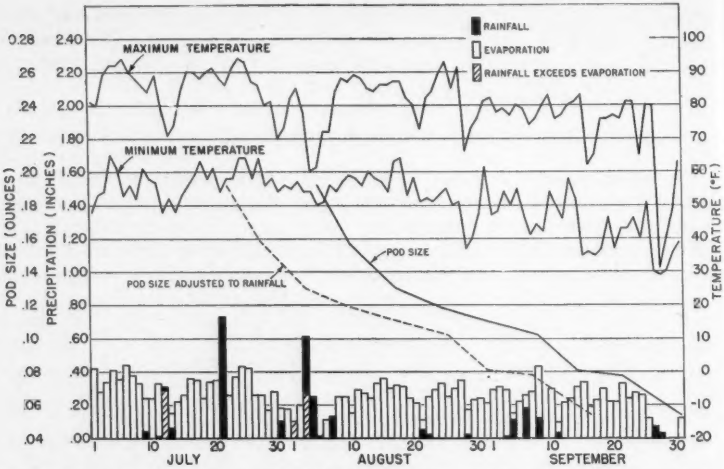

FIGURE 3.—Comparison of effects of maximum and minimum temperatures, evaporation, and precipitation on average pod size of 35 varieties and strains of snap beans, 1936.

Examination of figure 2, for 1935, appears at first glance to show but little correspondence between rainfall and pod size. However, a light irrigation given between the first and second harvest periods

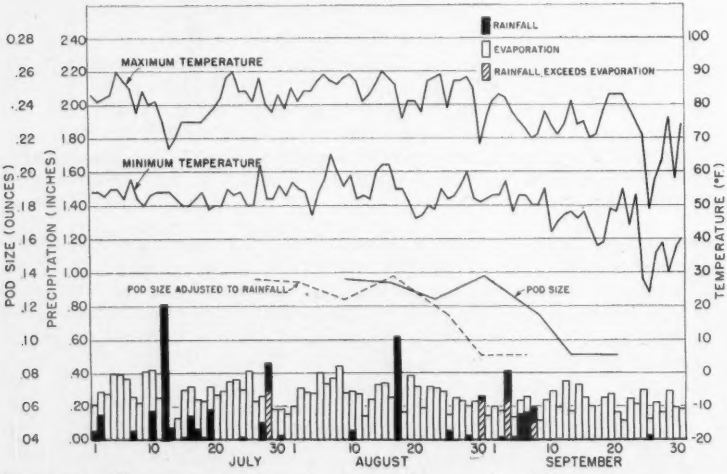

FIGURE 4.—Comparison of effects of maximum and minimum temperatures, evaporation, and precipitation on average pod size of 35 varieties and strains of snap beans, 1937.

was sufficient to cause a slight increase in pod size about 2 weeks later, and a rainy period from the 7th to the 9th of August further increased the upward trend. In this latter instance the lag in response of the plants did not exceed 10 days.

In 1936 (fig. 3) there was a rapid decrease in pod size from August 5, the date of the first harvest, to August 17, at which time the effects of the rainfall of August 3 and 4 became evident through a slight checking of the rapid decrease. From September 8 to 14 there was another period of rapid decrease in pod size, which was checked by the rains of September 4, 6, and 8. Thus, in the first instance there was a lag of approximately 14 days between the occurrence of rain and plant response as measured by a decrease in pod size, and in the second instance the effects of rain were evident in from 7 to 11 days.

In figure 4, for 1937, there is an almost perfect agreement between the adjusted line for pod size and the occurrence of rainfall. Thus,

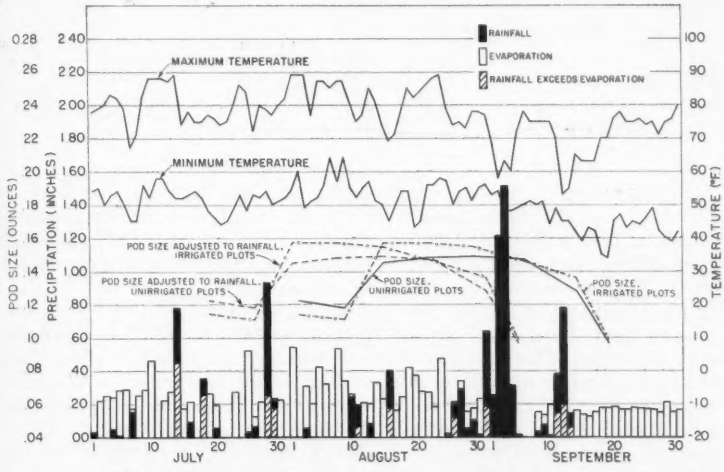

FIGURE 5.—Comparison of effects of maximum and minimum temperatures, evaporation, and precipitation on average pod size of 19 snap bean varieties grown under irrigation and under dry-land conditions, 1938.

the rainfall of August 17 had checked the rapid decrease in pod size by August 23, and by August 31 it had produced a measurable increase. Likewise, the rainfall of August 30 served to check a rapid decrease by September 13, and subsequent rains on September 3, 5, 6, and 7 prevented any further decrease to the end of the harvest season. From these data it appears that there was a lag of 7 to 14 days between a period of rainfall and response by the plants.

In figure 5, for 1938, it is possible to make a comparison of average pod size from irrigated and unirrigated plots. In this year, however, the rainfall of July, August, and September amounted to 9.74 inches, as compared with 3.21 inches in 1935, 2.97 inches in 1936, and 4.24 inches in 1937 (table 2). This amount of rainfall was evidently sufficient to promote almost normal growth, since additional water supplied through irrigation increased pod size to a measurable extent only at the third, fourth, and fifth harvest dates.

TABLE 2.—*Precipitation and evaporation at the United States Horticultural Field Station, Cheyenne, Wyo., 1935–38*

Month	Precipitation				Evaporation			
	1935	1936	1937	1938	1935	1936	1937	1938
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
January	0.10	0.14	0.34	0.17				
February	.39	.34	.17	.10				
March	.09	.62	1.70	1.21				
April	2.78	2.05	1.23	2.41				
May	5.57	2.19	1.91	2.74		6.721	6.197	6.771
June	2.92	2.61	2.41	2.42	6.693	8.505	6.743	6.480
July	.80	1.25	2.29	2.76	7.578	6.363	8.603	6.959
August	.36	1.17	1.00	2.28	8.363	7.331	8.596	7.087
September	2.05	.55	.95	4.70	5.443	6.687	6.218	6.956
Total for July, August, and September	3.21	2.97	4.24	9.74	21.384	20.381	23.417	21.002
October	.25	.58	1.02	.52				
November	.19	.21	.20	.42				
December	.03	.36	.79	.51				
Total for the year	15.53	12.07	14.01	20.24				

A comparison of the line representing pod size in 1938 with those of the other 3 years shows that it was much more level during a major portion of the harvest season. Coupled with the fact that there was a much more even distribution of rainfall in 1938 than in any of the other 3 years, this may be taken as added evidence that rainfall was the most important factor of those measured in determining pod size.

From these data it is impossible to demonstrate whether temperatures directly influence pod size or whether they are merely concomitant phenomena. It is fairly evident that temperature extremes are not consistently related to pod size, and so it may be assumed that their departure from the optimum range was not sufficient to be an important determining factor. General temperature trends, on the other hand, show a striking resemblance to those for changes in pod size. To this rule, however, there are several important exceptions, and it is believed that the available moisture supply rather than temperature is the important deciding factor.

TABLE 3.—*Annual average number of pods of snap beans per pound produced in tests at Cheyenne, Wyo., 1935–38*

[Listed in order of average performance for all years under test]

Varieties and strains	Average number of pods per pound				
	1935 (1 light irrigation)	1936 (no irrigation)	1937 (no irrigation)	1938	
				No irrigation	Normal irrigation
388–8–5	107.75	188.06	196.46		
367	103.67	172.83	159.92		
391–15–4	112.83	153.17	144.17		
344	96.00	154.78	149.08		
328	99.42	155.56	137.88		
391–15–2	99.17	145.94	146.38	131.42	130.83
280	90.25	160.67	140.46		
282	96.17	157.28	135.38		
308	100.50	154.28	128.67		
U. S. No. 5 Refugee	98.83	176.56	146.83	120.58	95.08

TABLE 3.—*Annual average number of pods of snap beans per pound produced in tests at Cheyenne, Wyo., 1935–38*—Continued

Varieties and strains	Average number of pods per pound				
	1935 (1 light irrigation)	1936 (no irrigation)	1937 (no irrigation)	1938	
				No irrigation	Normal irrigation
391–1–4	104.17	158.00	135.54	121.25	115.92
305	93.08	149.00	131.71	126.42	134.50
309	98.33	155.83	133.13	117.25	124.25
287	99.00	153.61	135.88	124.08	115.25
285	100.00	149.94	129.67		
U. S. No. 1 Refugee	96.08	150.22	129.04	123.25	125.17
388–1–5	83.17	176.44	146.04	108.58	106.17
283	94.42	147.89	129.88		
229	95.92	141.11	134.92		
286	93.00	151.72	131.54	121.50	122.08
304	95.75	148.61	131.75	123.25	116.75
284	98.83	149.67	132.04	117.67	116.25
Stringless Green Refugee	99.08	151.33	143.29	118.58	103.00
919	97.25	154.17	132.58	110.00	113.83
228	98.33	151.11	127.67	118.92	109.50
388–7–6	114.42	128.67	119.33		
391–15–11	89.75	133.72	127.13		
341	86.25	135.94	126.38		
388–6–2	87.00	131.89	121.58	120.00	110.75
342	83.00	130.89	125.54		
Brittle Wax	79.92	114.83	100.96	94.08	98.50
Giant Stringless Greenpod	74.67	114.44	103.50	99.17	91.67
Burpee Stringless Greenpod	86.33	106.89	94.04		
Full Measure	68.42	101.56	105.38	95.83	97.17
New Stringless Greenpod	66.67	95.00	104.42	103.33	92.33
Average for all varieties	93.93	145.76	131.05	115.53	111.53
Significant difference between varieties	11.71	14.45	9.91	15.49	15.49
Standard error	4.14	5.11	3.50	5.48	5.48

Table 3 shows as seasonal averages the number of pods per pound for the varieties and strains of beans tested each year. The order of listing these varieties and strains was determined by taking an average of their performances for all the years that they were under test. Thus, strain 388–8–5, which was under test for 3 years, produced on an average the greatest number of pods per pound, whereas New Stringless Greenpod produced the least number of pods per pound for the 4 years that it was under test.

Exact comparisons of the performances of these varieties and strains from year to year are not possible because of changes in experimental design that made a generalized analysis of the data for all years impossible. It is interesting to note, however, that the average number of pods per pound is greater for the dry seasons of 1936 and 1937 than it is for 1935, when one irrigation was given, or for 1938, the year of most abundant rainfall.

Comparisons between varieties and strains grown in any given year may be made with the aid of the values for significance appearing at the bottom of table 3. By such comparisons it is evident that in some varieties pod size is a fairly stable characteristic. This is especially true of the commercial varieties Brittle Wax, Giant Stringless Greenpod, Full Measure, New Stringless Greenpod, and Burpee Stringless Greenpod, which, with the exception of the last-named variety in 1935, consistently produced larger pods than most of the hybrid strains or than U. S. No. 1 Refugee or U. S. No. 5 Refugee.

On the other hand, such unstable strains as 344, 285, 280, 388–1–5, or 388–7–6 showed relatively much greater variability from year to year. Except in 1937, U. S. No. 1 Refugee did not differ significantly in pod size from Stringless Green Refugee; but U. S. No. 5 Refugee was more variable, as may be seen by its much greater reduction in size under conditions of extreme drought such as existed in 1936 and 1937.

As has already been stated, counts were made of the number of pods per pound at each harvest date; and though it did not seem worth while to analyze these data for each date, an analysis was made that included an early, a midseason, and a late harvest. This analysis showed that there was a highly significant interaction between varieties and periods for the years 1935, 1936, and 1937. In each case the F value exceeded the 1-percent point. In the interest of brevity, only the data for 1935 are shown in detail (table 4). By the aid of the values appended to table 4 it is possible to measure the significance of the various interactions and that of the differences between and within varieties at each harvest period.

TABLE 4.—*Number of pods of snap beans per pound at early, midseason, and late harvest dates in tests at Cheyenne, Wyo., 1935*

Varieties and strains	Early harvest	Mid-season harvest	Late harvest	Varieties and strains	Early harvest	Mid-season harvest	Late harvest
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
388–8–5	101.50	112.75	109.00	284	74.00	115.00	107.50
367	103.25	109.25	98.50	Stringless Green Refugee	90.25	115.25	91.75
391–15–4	102.50	120.50	115.50	919	77.25	102.25	112.25
344	95.75	103.75	88.50	228	73.75	103.25	118.00
328	87.00	105.75	105.50	388–7–6	104.00	120.00	119.25
391–15–2	88.75	104.50	104.25	391–15–11	84.00	98.00	87.25
280	74.50	99.75	96.50	341	72.25	83.50	103.00
282	79.75	102.25	106.50	388–6–2	78.00	89.00	94.00
308	81.75	109.25	110.50	342	69.00	88.25	91.75
U. S. No. 5 Refugee	84.25	97.25	115.00	Brittle Wax	59.25	75.00	105.50
391–1–4	88.50	111.75	112.25	Giant Stringless Greenpod	59.75	77.75	86.50
305	78.50	98.00	102.75	Burpee Stringless Greenpod	65.25	73.75	120.00
309	77.00	93.50	124.50	Full Measure	58.00	66.50	80.75
287	82.25	111.75	103.00	New Stringless Greenpod	59.50	77.50	63.00
285	83.25	108.00	108.75				
U. S. No. 1 Refugee	84.00	106.00	98.25				
388–1–5	78.75	85.25	85.50				
283	80.75	98.25	104.25				
229	79.75	108.50	99.50				
286	82.50	100.50	96.00	Average	80.71	99.33	101.74
304	86.25	105.00	96.00				

Value for significance between averages for periods, 3.34; standard error, 1.18.
Value for significance between any two means in the table, 20.29; standard error, 7.17.
Value for significance for interaction "strains × periods," 28.69.

From the data given it will be seen that 12 varieties and strains produced a greater number of pods per pound at the midseason than at the early harvest, and none produced less. Stringless Green Refugee shows a significant decrease in number of pods per pound between the midseason and late harvest periods, whereas No. 309, Brittle Wax, and Burpee Stringless Greenpod show an increase. Most of the interactions implied by these differences have been found to be significant.

SUMMARY

During a 4-year period certain commercial varieties of snap beans were compared with U. S. No. 1 Refugee and U. S. No. 5 Refugee beans and several related strains to determine variations in their relative drought tolerance.

Under the conditions of these tests it was shown that U. S. No. 1 Refugee and U. S. No. 5 Refugee and certain hybrid strains possessed the ability to yield better under drought or semidrought conditions than the commercial varieties tested.

In 1938 the appearance of bacterial blight (*Bacterium phaseoli* E. F. Smith and *Bact. medicaginis* var. *phaseolicola* Burk.) complicated to some extent the determination of drought effects.

A study of the relative damage caused by bacterial blight on the 19 varieties and strains under test in the dry-land plots showed that U. S. No. 5 Refugee and Full Measure were the least injured, though Stringless Green Refugee was not significantly different. All other varieties and strains showed significantly greater damage than the first two varieties. In the irrigated plots U. S. No. 5 Refugee showed the least injury, and 10 varieties and strains were significantly more damaged.

A comparison of the effects of maximum and minimum temperatures, evaporation, and rainfall on pod size showed that rainfall was the most important determining factor. In general, there was a lapse of from 7 to 14 days from the time that rain fell until there was a measurable difference in pod size.

A majority of the varieties and strains under test showed a significant decrease in pod size whenever available soil moisture became a limiting factor. Certain of them, however, showed an actual increase in pod size.

In general, the commercial varieties were more stable with respect to pod size than the hybrid strains.

MOTTLE LEAF, A VIRUS DISEASE OF CHERRIES [1]

By E. L. REEVES [2]

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INTRODUCTION

A graft-transmissible disease of cherry trees herein referred to as mottle leaf is known to have existed in different sections of the Pacific Northwest for the last several years. In 1917 D. F. Fisher photographed [3] cherry leaf specimens collected near Monitor, Wash., which exhibited symptoms similar to mottle leaf. Zeller (*8*) [4] reported having observed the disease in 1920 on Napoleon (Royal Ann) cherry trees growing in Oregon. Leaf specimens exhibiting typical mottle leaf symptoms were observed in the collection of Dr. C. W. Hungerford, University of Idaho, from Bing cherry trees growing in the Lewiston (Idaho) district, and collected September 1, 1922. Cherry leaves collected by B. F. Dana near Kennewick, Wash., during the summer of 1924 and deposited in the collections of the Department of Plant Pathology, State College of Washington, have also been examined, and these apparently represent typical mottle leaf. Several orchardists near Wenatchee, Wash., have given interesting accounts of the early appearance of peculiar malformed leaves on their cherry trees, and it is considered probable that as early as 1910 a few cherry trees growing in the north central part of Washington were affected by mottle leaf. At the present time sweet cherry trees growing in Washington, Oregon, Idaho, and British Columbia have been found to be affected by the disorder. In California, one Waterhouse cherry tree was observed that exhibited symptoms considered typical of mottle leaf.

Preliminary investigations reported in 1935 by McLarty [5] and by Reeves (*6*) demonstrated that mottle leaf was a graft-transmissible disease of sweet cherries. Previously, the disorder had been vaguely attributed to various factors such as unfavorable soil conditions, severe spring frosts, rosette or little leaf, poor root systems, chlorosis, and crown gall. Subsequent observations and transmission tests have definitely indicated that the disease is of virus origin and also have revealed that variations of symptom expression occur on different varieties of both sweet and sour cherries. Mottle leaf has been observed occurring naturally under field conditions on the sweet cherry (*Prunus avium* L.) and the wild cherry (*P. emarginata* (Dougl.) Walp.), but it has been transmitted to other species of *Prunus*. The

[1] Received for publication December 31, 1940. The material presented herein represents the substance of a thesis submitted to the faculty of the Graduate School of the State College of Washington in partial fulfillment of the requirements for the degree of master of science.

[2] Special appreciation is expressed to C. P. Harley, of this Division, and to Dr. F. D. Heald, of the State College of Washington, for advice and suggestions during the course of this work; and to Dr. Lee M. Hutchins and Dr. John W. Roberts, of this Division, for helpful criticism of the manuscript.

[3] Unpublished photograph is in possession of author.

[4] Italic numbers in parentheses refer to Literature Cited, p. 571.

[5] MCLARTY, H. R. CHERRY MOTTLE LEAF. Northwest Assoc. Hort., Ent. and Plant Path., Abs. Papesr 1: 5. 1935. [Mimeographed.]

purpose of this paper is to present the results of studies on symptoms and transmission of cherry mottle leaf, together with incidental data gathered during the course of the investigation.

LEAF SYMPTOMS

The leaves of cherry trees affected by mottle leaf exhibit the most distinctive symptoms of the disorder, although a considerable variation in the intensity of the leaf symptoms exists among different cherry varieties and to some extent among different trees of the same variety. In the Wenatchee, Wash., district, where most of the observations have been made, the most commonly affected variety is the Bing. This variety is important commercially in the Pacific Northwest, particularly in the State of Washington, where Bing constitutes approximately 58 percent of all sweet cherry trees according to a tree survey made in 1936.[6]

The leaves from a severely affected Bing tree shown in figure 1, *A*, exhibit the mottling of color and puckered leaf condition characteristic of the disorder. The periphery of the leaves is irregular, and midvein and lateral veins failed to develop as in a normal leaf, with the result that the leaf has become puckered and misshapen. Light-yellow or yellowish-green areas and spots appear in the interveinal tissue and along the veins. These chlorotic areas become apparent soon after the leaves unfold in the spring, and produce the conspicuous mottling that on the Bing variety remains evident throughout the period of foliation. No retarded foliation of diseased trees has been observed, and the leaves do not fall prematurely. Some of the spots and irregular light-colored areas may drop out, leaving holes or irregular perforations in the leaf. The chlorotic areas are quite translucent in transmitted light, in contrast to the nearly opaque green tissue of the leaf. Practically all leaves on a severely diseased Bing tree exhibit symptoms and on the average are smaller than the leaves of a healthy tree. Figure 1, *B*, illustrates an extreme difference in size between leaves from a healthy tree and leaves from a severely diseased tree, but the average difference is striking enough to be noticeable. When one becomes familiar with mottle leaf symptoms, affected trees may be recognized from a considerable distance because of the abnormal appearance of the foliage.

Certain variations in leaf symptoms may occur, particularly during the first year that a tree is naturally affected by mottle leaf. Examples of such variations are shown in figure 2. In *A* the leaf shows some ruffling and little mottling of color. In *B* and *C* the leaves exhibit no rugosity, but the chlorotic areas are plainly evident, approximating a veinlet clearing in *B* to a distinct chlorosis along the veinlets in *C*. In *D* the leaf is almost entirely chlorotic in appearance and slightly ruffled. The leaves of an average diseased Bing tree usually exhibit symptoms ranging from moderate (fig. 2, *E*) to the more intense or severe symptoms shown in figure 1, *A*. In figure 2, *F*, the leaf is from a healthy Bing tree. All the leaves in figure 2 showing symptoms were taken from naturally infected trees. Leaves with symptoms considered typical of mottle leaf are usually in greater abundance on a tree than those with atypical symptoms. In instances where

[6] HEISIG, C. P. WASHINGTON TREE FRUIT AND BERRY ACREAGE SURVEY. Wash. Agr. Expt. Sta. 1937. Mimeographed.]

diseased trees exhibit leaf symptoms similar to those in figure 2, *C* and *D*, the question might be raised as to whether some virus other than the infective principle responsible for typical mottle leaf

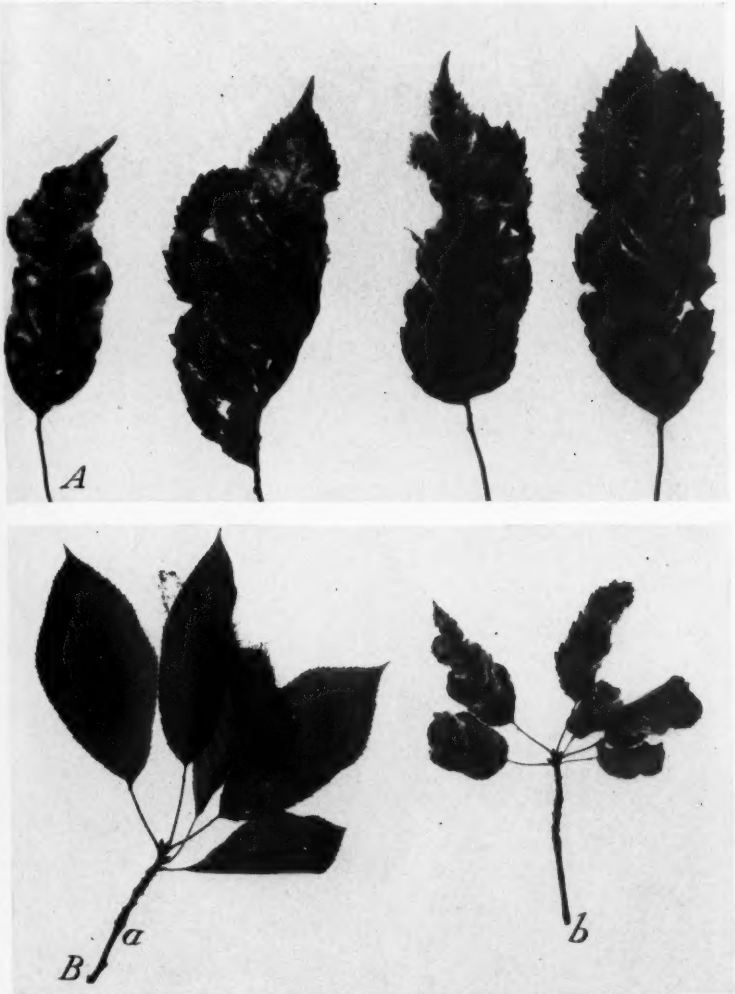

FIGURE 1.—Leaves from naturally infected Bing cherry trees, showing severe mottle leaf symptoms. *A*, Four leaves exhibiting the mottling of color and puckering characteristic of the disorder. About four-fifths natural size. (Photographed by transmitted light.) *B*, Twigs from a healthy (*a*) and from a diseased (*b*) Bing cherry tree, showing the difference in leaf size. About one-third natural size. (Photographed by transmitted and reflected light.)

might be responsible for the variable symptoms. This question cannot be definitely answered, although results of transmission tests up to the present time suggest that only one virus is involved, since

variable leaf symptoms have not appeared on graft-inoculated trees.

The first year a tree is affected by mottle leaf, the symptoms frequently appear only on leaves of a few branches. Usually in the

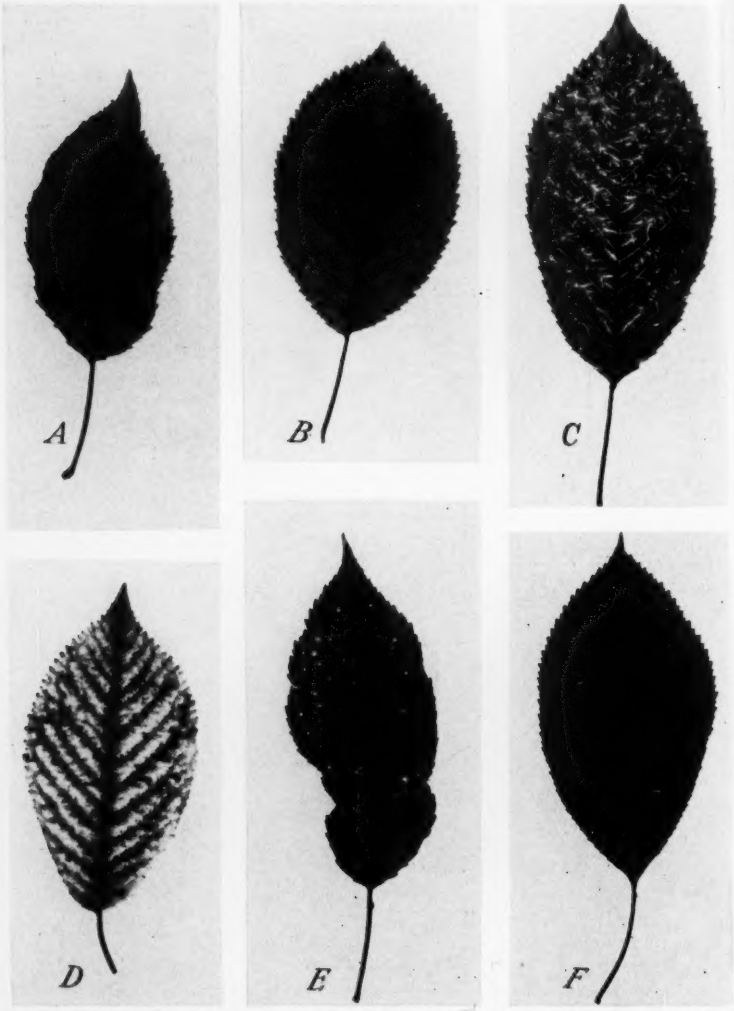

FIGURE 2.—*A–D*, Symptom variations of cherry mottle leaf; *E*, representative of mottle leaf symptoms of moderate intensity; *F*, leaf from a healthy tree. All leaves from Bing trees. See text for discussion. (Photographed by transmitted light.)

second year the majority of leaves are mottled and the symptoms become more pronounced; the leaves often decrease in size during succeeding years.

A few trees have been observed in which leaf symptoms were noticeably less intense than in preceding years. This decrease cannot be accounted for except, perhaps, on the basis of more favorable cultural and growing conditions. No tree has been known to recover from mottle leaf, and any decrease in severity of symptoms has not been sufficient to cause doubt as to the identity of the disorder.

EFFECT OF MOTTLE LEAF ON GROWTH AND FRUIT OF TREE

Studies on the effect of mottle leaf on the growth and fruit of the tree have been directed mainly to the Bing and Napoleon varieties. In general, the extent to which growth and fruit of the tree were affected could be correlated with the severity of leaf symptoms, the length of time the tree had been diseased, and the size of the fruit crop.

The terminal growth of diseased trees was less than that of healthy trees growing under similar cultural conditions. The first year a tree was affected there was little or no effect on the growth. After 2 or more years the reduction in terminal growth was especially noticeable if the leaf symptoms were severe. In advanced stages of the disease, the spurs made short growths and there was a general stunting of the tree.

Blossoming dates and the character of the blossoms have apparently not been influenced by mottle leaf, and there was little or no difference in the quality of the fruit the first year a tree exhibited leaf symptoms. Trees bearing a heavy crop of fruit and known to be severely affected by mottle leaf for 3 or more years have been found to produce but little marketable fruit, or none at all. The fruit on such trees was usually undersized, insipid, and late in ripening. After 2 or 3 years the disease evidently influences leaf function to the extent that a heavy crop of fruit cannot mature properly. When bearing only a light crop, a tree affected by mottle leaf may produce fruits of nearly normal size that are only slightly insipid. In general, however, Bing and Napoleon trees affected by mottle leaf for more than 2 years have not produced a commercial crop of fruit. Occasionally small, irregular, light-brown necrotic areas developed on the fruits of severely diseased Napoleon trees 3 or 4 weeks before harvest. As the fruit approached maturity the necrotic areas became slightly depressed, causing the fruit to become misshapen. These necrotic areas do not necessarily develop on the fruits of an affected tree in successive years, and factors other than the disease may be partly or wholly responsible for the condition.

VARIETAL SUSCEPTIBILITY

OCCURRENCE OF MOTTLE LEAF UNDER FIELD CONDITIONS

Under field conditions in the Pacific Northwest, only four varieties of sweet cherries (*Prunus avium* L.) have been found with mottle leaf symptoms. The Bing is most often affected, and because this variety exhibits definite and severe leaf symptoms it was selected as the test variety for experimental work. Naturally affected trees of the Napoleon (Royal Ann), a variety that constitutes approximately 17 percent [7] of the commercially planted sweet cherry trees in Washing-

[7] See footnote 6.

ton, often show leaf symptoms equally as severe as are shown by trees of the Bing variety. The Waterhouse or Long Stem Waterhouse, a rather unimportant variety commercially, also exhibits definite leaf symptoms. The fourth variety found affected under field conditions is the Lambert, which constitutes approximately 18 percent [7] of the commercially planted sweet cherries in Washington. However, only three Lambert trees with mottle leaf symptoms, all located in one orchard near Wenatchee, have been discovered. A comparatively few leaves on each tree were affected, and the symptoms were attenuated and somewhat fugitive. Moderate interveinal chlorosis, accompanied by a slight leaf ruffling (fig. 3), was found on

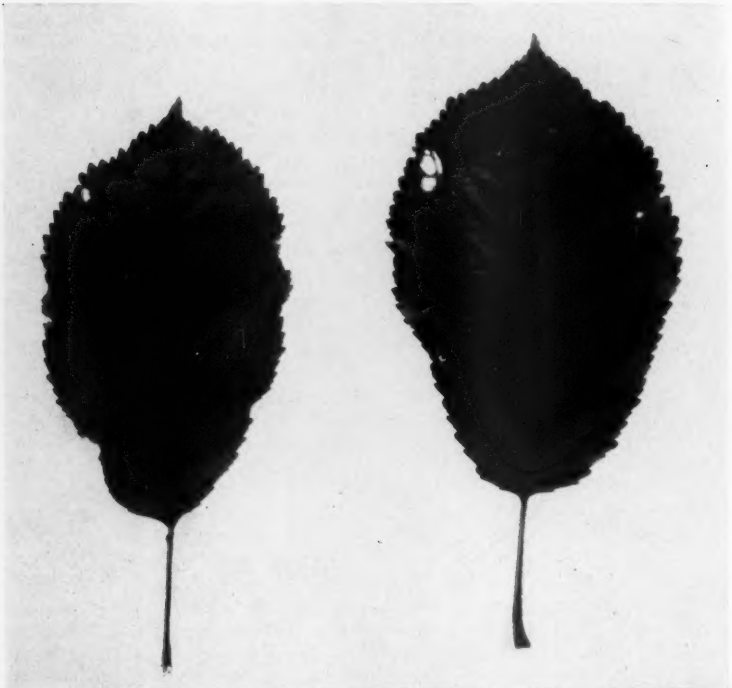

FIGURE 3.—Symptoms of mottle leaf in Lambert cherry, showing moderate interveinal chlorosis and slight leaf ruffling. (Photographed by transmitted and reflected light.)

about 20 percent of the leaves 5 to 6 weeks after growth started in the spring. The chlorosis was evident until shortly after harvest and then gradually disappeared, although the leaf ruffling persisted for some time. With these exceptions Lambert was found to be quite free from symptom expression.

TRANSMISSION EXPERIMENTS

In order to determine leaf symptoms of mottle leaf on varieties apparently free from the condition in the field, two methods of procedure

[7] See footnote 6.

were employed: (1) Buds of different cherry varieties were budded into diseased Bing trees, and (2) buds from diseased Bing trees were budded into healthy young trees of different varieties. In instances where leaf symptoms were not clearly discernible on the test trees infected by budding, buds were taken from such trees and placed in healthy young Bing trees to prove the presence of the virus in tissues of the variety so tested. No variety proved to be immune, although several varieties were highly tolerant and practically symptomless.

Eleven varieties of sweet cherries were tested by budding into diseased Bing trees, with the result that four varieties—Bing, Napoleon, Waterhouse, and Centennial—exhibited severe leaf symptoms. Somewhat variable symptom expression was obtained for Black Tartarian, Republican, Early Purple, and Governor Wood varieties, but the leaves were definitely ruffled and in a few instances chlorotic spots appeared. However, the mottling of these four varieties was mostly fugitive and was entirely absent from many trees. The leaf symptoms for the Deacon and Parkhill Seedling varieties proved to be much the same as for the Lambert described above.

Three varieties of sour cherries (*Prunus cerasus* L.) were tested and all proved tolerant to mottle leaf. The Montmorency and Early Richmond varieties exhibited occasional chlorotic spots on a few leaves, but no leaf ruffling was observed. No symptoms of any kind were found on leaves of English Morello.

Three varieties of duke cherries (*Prunus avium* × *P. cerasus*) were tested and are considered tolerant to mottle leaf. Occasional leaves of the May Duke variety exhibited light mottling for a short time and were also slightly ruffled. Practically no leaf mottling was observed on either Late Duke or McKee, and there was only occasional slight ruffling of a few leaves.

Mahaleb trees (*Prunus mahaleb* L.) grown from seed and inoculated with mottle leaf by budding showed practically no leaf symptoms. However, when buds from healthy mahaleb trees were budded into diseased Bing trees, a few chlorotic spots and slight leaf curling developed on about 10 percent of the leaves on the growth from the mahaleb buds. The mahaleb was therefore considered partially tolerant to mottle leaf.

Wild cherry trees (*Prunus emarginata*) growing in their native habitat and budded with diseased Bing buds produced leaves with variable mottling and light leaf ruffling. When buds were taken from the wild cherry and budded into diseased Bing trees, a few of the wild cherry leaves showed light ruffling and a change of color approximating interveinal chlorosis. In 1936 leaves of somewhat similar appearance were observed on wild cherry trees growing under natural conditions (fig. 4). When buds taken from these trees were inserted into healthy Bing trees severe mottling characteristic of mottle leaf resulted. Some of the wild cherry trees on which the disease occurred naturally were growing within 40 feet of a mottled Bing tree. This would suggest a possible relationship between the disease on the Bing and that on the wild cherry because of their proximity. This particular situation indicates the possibility of an insect vector attacking both the sweet cherry and the native wild cherry, which may have an important bearing on the natural spread of the disease. In the north central part of Washington, it has been

commonly observed that trees affected with mottle leaf are more prevalent in orchards planted in canyons and in foothill orchards near which native wild cherry trees are growing.

Table 1 presents a summary of the leaf symptoms of mottle leaf exhibited by different species and varieties of cherries, with remarks concerning the relative intensity of symptom manifestation in the leaves. Since observations for some varieties were made on young trees and since leaf symptoms for a variety may be variable, this table will be subject to additions and revision as additional information is obtained.

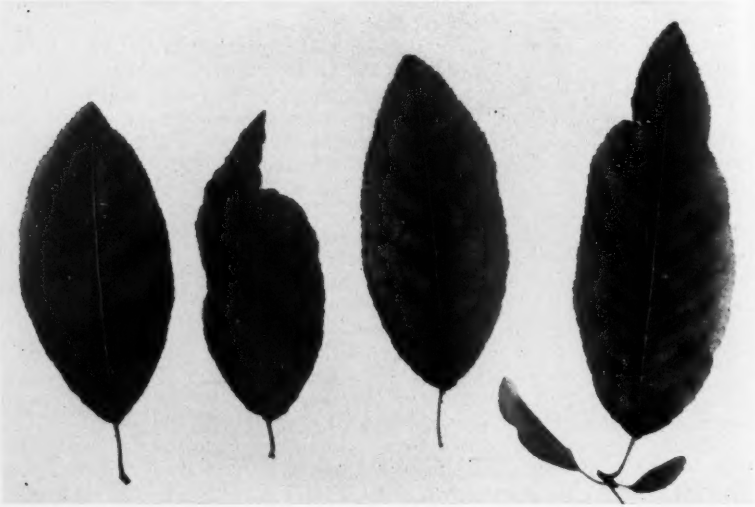

FIGURE 4.—Symptoms of mottle leaf in a naturally infected native wild cherry (*Prunus emarginata*). Normal leaf at left. (Photographed by transmitted light.) Healthy Bing trees inoculated by budding from such wild cherry trees produced leaves with very severe mottle leaf symptoms similar to those illustrated in figure 1, *B*.

Attempts were made to transmit mottle leaf to different varieties of plum, peach, and apricot. Buds from diseased Bing trees were inserted into Climax plum, Italian Prune plum, Double × Agen (French Petite) plum, Late Crawford peach, Triumph peach, Tilton apricot, Wenatchee Moorpark apricot, and Riland apricot. Five young trees of each variety were used in the experiment; two infective buds were inserted into each tree in 1936, and three or more buds in 1937. None of the buds lived, and upon examination no evidence was found of tissue union. Since no tissue union was established between diseased and healthy tissue, all trees remained free from mottle leaf. These tests, therefore, failed to show whether or not plums, peaches, and apricots are susceptible.

In 1939, under greenhouse conditions, healthy Bing scions were successfully grafted on branches of three J. H. Hale peach trees. Buds from diseased Bing trees were then inserted into trunks of the peach trees, and growth union was established. Typical mottle leaf

symptoms appeared on leaves of the Bing scions, which demonstrated that the virus had passed through the tissues of the peach. A few of the peach leaves also exhibited a slight mottling.

TABLE 1.—*Relative intensity of mottle leaf symptoms in species and varieties of cherries*

Species of cherry	Variety	Leaf symptoms	
		Mottling	Rugosity
Prunus avium	Bing	Pronounced	Severely puckered.
	Napoleon	do	Do.
	Waterhouse	Moderate	Puckered.
	Centennial	do	Do.
	Black Tartarian	Slight, variable	Moderately ruffled.
	Early Purple	do	Do.
	Governor Wood	do	Do.
	Republican	do	Do.
	Lambert	Slight, fugitive	Lightly ruffled.
	Deacon	do	Do.
	Parkhill seedling	do	Do.
P. cerasus	Montmorency	Occasional slight	None.
	Early Richmond	do	Do.
	English Morello	None	Do.
P. avium × *P. cerasus*	May Duke	Occasional, slight	Lightly ruffled.
	Late Duke	Very slight, fugitive	Occasional light ruffling.
	McKee	do	Do.
P. mahaleb	Mahaleb	Occasional, slight	Do.
P. emarginata	Wild cherry	Medium, fugitive	Do.

As previously mentioned, preliminary transmission tests, started in 1934 and reported in 1935 (*6*), indicated that mottle leaf could be transmitted from diseased to healthy cherry trees by the ordinary methods of budding or grafting; and further tests have demonstrated that the disease could be transmitted by any means where growth union was established between diseased and healthy tissues. In August 1935, buds from diseased trees were budded into 27 healthy trees of 9 different varieties of sweet and sour cherries. The infective buds remained in good condition until late in October 1935, when early cold weather killed all the buds and seriously injured most of the trees. With the exception of one tree, which was winter-killed, all trees later proved to be infected with mottle leaf. Later tests showed that any "patch-bark" graft was effective in transmitting the disease whenever growth union of tissues was established.

The time necessary for the development of leaf symptoms after artificial bud or graft inoculation apparently varies with different conditions. A scion from a diseased Bing tree was tongue-grafted on a small branch of a young healthy Napoleon tree March 28, 1935, before growth started. The first leaves coming out on the scion showed typical mottle leaf symptoms. On June 20, 1935, 84 days after the graft was made, the terminal leaves on an adjacent Napoleon shoot exhibited symptoms of mottle leaf (fig. 5). Later in the season mottle leaf also appeared on leaves of the current season's growth on other branches of the main lateral limb on which the inoculating scion had been grafted. Leaf symptoms did not appear on the leaves of other main lateral limbs until 1936.

In an experiment designed to test transmission of mottle leaf through the roots, an old diseased tree was cut down and roots ½ to 1½ inches in diameter were uncovered. On April 28, 1939, 20 young

healthy Bing trees were root-grafted to the roots of the diseased tree, and 7 showed definite leaf symptoms 60 days after the grafts were made. Later, all the trees that formed a growth union with the roots of the diseased tree showed mottle leaf symptoms.

In the following experiment, symptoms were obtained 37 days after infective buds were placed in healthy trees. Bud sticks taken from a diseased Bing tree in January 1937 were held in 32° F. storage until

FIGURE 5.—Spread of mottle leaf on a Napoleon cherry tree. On March 28, 1935, a scion from a diseased Bing tree was tongue-grafted on the small upper branch at the point indicated by arrow. All leaves on the scion show symptoms. A leaf on the lower branch, the tip of which is held by hand, was the first leaf of the newly infected tree to show symptoms. (Photographed July 2, 1935.)

June 5, 1937, and at this time the buds were inserted into healthy young Bing trees. On July 12, 1937, mottle leaf symptoms were observed on leaves of two of the seven trees used in the test. Under greenhouse conditions the time necessary for the development of leaf symptoms after bud inoculation was considerably shortened. When healthy young Bing trees growing in the greenhouse were so inoculated, faint leaf symptoms occurred on a few leaves in 9 days, and the same leaves showed definite mottle leaf 14 days after budding. Under field conditions, budding or grafting in midsummer or later failed to produce symptoms of the disease until the following spring.

Evidence that the infective principle of mottle leaf is transported in the tree by conductive tissues of the phloem was obtained by ringing experiments. A ring of bark about 4 to 6 mm. in width was removed from the trunk of each of 10 young Bing trees, from 3 to 4 feet in height, and diseased buds were inserted above or below the ring. Mottle leaf symptoms developed on the side of the ring where the infective bud was inserted, but in no instance did symptoms develop on the opposite side of the ring.

All mechanical methods of transmission employed, where no growth union of tissue was established, have been unsuccessful in transmitting mottle leaf. Many mechanical methods of transmitting virus diseases as used by other investigators, or adaptations thereof, have been employed in an attempt to transmit mottle leaf. Juice from diseased plants was obtained by grinding either leaves or tender stem tissues or both in a mortar and pressing the juice through cheesecloth. In some instances a small amount of distilled water was added during the grinding process. The juice thus obtained was used in different ways in an attempt to inoculate healthy cherry trees. The four principal methods employed were (1) injection of juice into the base of leaf petioles and tender stem tissue by means of a fine hypodermic needle; (2) the use of carborundum in applying juice to leaves as employed by Rawlins and Tompkins (*5*) except that a 400-mesh instead of a 600-mesh grade carborundum was employed; (3) rubbing leaves with a piece of cheesecloth saturated with juice without employing carborundum; and (4) placing a drop of juice on the leaf to be inoculated and pricking through the drop with a fine needle. Other methods employed were (1) binding together diseased and healthy leaves and pricking through them with fine needles; (2) holding a diseased leaf against the stem of a healthy plant and pricking the leaf with fine needles; and (3) cutting into stem tissue of a healthy plant with a knife used to cut stems or leaves of a diseased tree. No indication was obtained that transmission of mottle leaf was effected by any of the mechanical methods employed.

SPREAD OF MOTTLE LEAF IN THE FIELD

The manner in which mottle leaf of cherries spreads in the field is as yet unknown. It is considered probable, however, that an insect vector is responsible for natural mottle leaf infections. Only one insect, the black cherry aphid (*Myzus cerasi* (F.)), was tested as a possible vector in the present study. No evidence of transmission was obtained.

There was considerable variation in the rapidity with which the disease spread in different orchards under observation in the north-central part of Washington. In several orchards no new infections have occurred during the past 4 years, but, on the other hand, new infections have increased rapidly in a few. For example, in one small planting of 84 cherry trees, 6 showed mottle leaf in 1935; 1 additional tree developed leaf symptoms in 1936; no new infections occurred in 1937; then 21 additional trees showed leaf symptoms in 1938. Out of 52 cherry orchards in the north-central part of Washington, in which one or more diseased trees were located, the spread of the disorder has been considered serious in only 7 during the last 4 years.

ATTEMPTS TO INACTIVATE THE VIRUS BY HOT-WATER TREATMENT

Following essentially the method employed by Kunkel (*2*), bud sticks from mottle leaf trees were subjected to hot-water treatments in an attempt to inactivate the virus. Bud sticks about 6 inches long and three-sixteenths to one-fourth of an inch in diameter were collected in January 1937 and held in cold storage until treated in April. Immediately after treatment the buds were inserted into healthy young cherry trees, and a scion with two buds was also tongue-grafted on each tree. The bud sticks were immersed in water held at the indicated temperatures for the following periods: (1) 41° C. for 15-, 60-, and 180-minute periods; (2) 46° for 10-, 30-, and 60-minute periods; and (3) 49° for a 10-minute period. Transmission of mottle leaf was obtained in all tests except by the buds treated at 41° for 180 minutes, in which instance no tissue union was established between the infective buds and the healthy trees. The buds subjected to 46° for 60 minutes and at 49° for 10 minutes were seriously weakened and ultimately died, although most of them lived about 3 weeks after budding, during which time tissue union was established. The experiment indicated that treatments severe enough to inactivate the virus would also kill the buds; however, a more extended range of temperature and treatment periods should be employed to definitely determine this. It is also recognized that different results might be obtained from similar experiments with budwood taken in July or August during the growing season, or perhaps a dry-heat treatment might be used that would inactivate the virus but would not kill the treated budwood.

CHERRY DISORDERS SOMETIMES CONFUSED WITH MOTTLE LEAF

Certain disorders of cherry trees that cause leaf malformations have sometimes been confused with mottle leaf. For the purpose of comparing them with mottle leaf, brief mention will be made of those most commonly found in Washington cherry orchards.

Malformed leaves typical of those found on "unproductive-type" cherry trees in California (*1*), the condition sometimes referred to as "crinkle," have been observed on several trees in Washington and Idaho. Most of the affected trees in Washington had malformed leaves on only a few branches, although an occasional tree was found with practically all leaves more or less affected. Symptoms of the condition have been described by Kinman (*1*) and will not be further discussed here. Figure 6 shows typical malformed leaves from a Bing tree that differ considerably in appearance from leaves of a tree affected by mottle leaf.

Another disorder of cherry trees exhibiting a definite leaf symptom but differing from mottle leaf was seen on a few Bing trees near Wenatchee, Wash. (*7*). Leaf symptoms, which do not become evident until after the leaves are fully expanded, first appear as faint chlorotic spots or areas and rapidly become more definitely yellow (fig. 7). The most seriously mottled leaves soon take on late-season colors, form abscission layers, and shed. This leaf fall occurs 2 or 3 weeks prior to harvest, affecting from 30 to 50 percent of the leaves. This does not take place in trees affected by mottle leaf. After leaf fall

the remaining foliage appears somewhat wilted and the leaf spots are often edged by darker yellowish-brown areas, producing a somewhat rusty appearance. This disease, referred to in field notes as "rusty mottle" to distinguish it from other disorders, has been repeatedly transmitted to healthy trees by budding. Affected trees apparently have normal blossoms, and the fruit is normal in shape although its flavor is insipid. The growth of affected trees was fair but slightly less than that of comparable healthy trees.

Another unnamed disorder was found in 1938 on Bing and Lambert cherry trees in three different orchards in the Wenatchee district. The symptoms exhibited by the leaves were considered different from those of other cherry disorders causing leaf malformations. The leaves were characteristically elongate (fig. 8), with the lateral veins

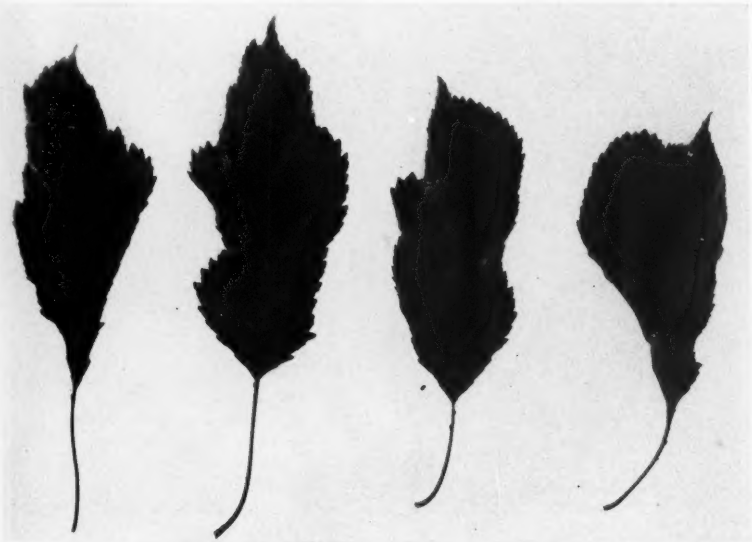

FIGURE 6.—Four malformed leaves from an unproductive type of Bing cherry tree. This leaf deformity is sometimes referred to as "crinkle." (Photographed by transmitted light.)

not fully developed on one side, and often both sides, of the midvein. The periphery of some leaves was quite irregular and perforations occurred on a few. A faint interveinal chlorosis was present on some leaves during the late spring and early summer, but was not so evident later in the season. The surface of affected leaves was quite rough or ruffled, but not puckered as in mottle leaf. Usually from 25 to 50 percent of the leaves on a tree were malformed and the remainder were normal or nearly so. The symptoms somewhat resemble those of the condition referred to as "crinkle," but differ principally in that the affected leaves do not have the pronounced chlorotic areas near the leaf margin, are more elongated, and have a rough surface (fig. 8). The fruit was definitely affected on some of the trees, but little on

others. The fruits usually had a pronounced depression on the suture side, and for that reason the disorder has been referred to as "deep suture." No information has yet been obtained regarding the transmission of the disorder.

FIGURE 7.—Leaf symptoms of a naturally infected Bing cherry tree caused by an unnamed graft-transmissible disease, tentatively referred to as "rusty mottle." This disorder is considered different from mottle leaf. (Photographed by transmitted light.)

Another apparently different disorder on Bing, Napoleon, and Deacon varieties was found in four orchards in the Wenatchee district. The condition has been referred to in field notes as "lace leaf," because of the unusual number of leaf perforations that develop and

take on the general appearance of pieces of lace. No information has been obtained concerning the possible causes of the condition, although it is apparently not associated with unusual cultural or growing conditions.

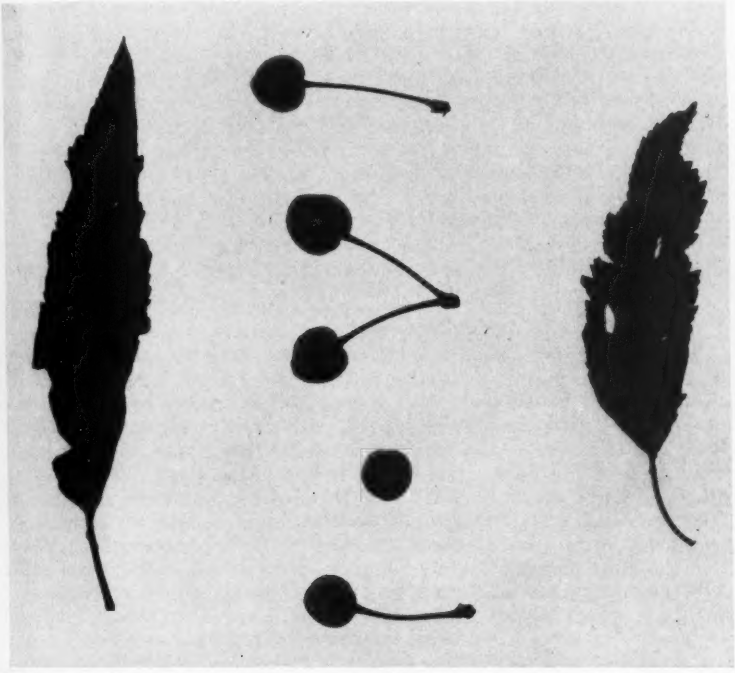

FIGURE 8.—Leaf and fruit symptoms on a Bing cherry tree caused by an unnamed disorder. Note the short lateral veins and unusual length of the leaves. Approximately one-half natural size. (Photographed by transmitted light.)

DISCUSSION

As was pointed out in the description of symptoms, there is considerable variability in the severity of the mottle leaf disease as indicated by the intensity of leaf symptoms during the first year a tree becomes affected. Healthy young Bing trees inoculated by buds or grafts exhibit approximately the same severity of leaf symptoms whether the infective tissue originated from a tree that had shown mottle leaf for many years or from one that had shown less severe symptoms for only 1 year. However, when the infective tissue originated from a naturally infected wild cherry tree, the leaf symptoms of inoculated young Bing trees were particularly severe, the resulting new shoots being quite small, severely mottled, and puckered. The growth of the young trees inoculated with the wild cherry mottle leaf were quite stunted. Observation of the inoculated trees for 2 years indicated that the disorder affecting the wild cherry was not only mottle

leaf, but a virulent form of the disease as judged by leaf symptoms of the inoculated trees. Whether the apparent virulence of mottle leaf can be increased by inoculating wild cherry trees with the disease from a Bing tree, and then inoculating a healthy Bing from the infected wild cherry tree has not yet been determined.

The presence of naturally infected wild cherry trees growing relatively near diseased Bing trees gave no indication as to which species first became infected. During 1937 and 1938 a large number of wild cherry trees growing in locations far removed from cultivated orchards were inspected for mottle leaf. As a result of this survey, wild cherry trees were found in two isolated localities with leaves having symptoms closely resembling those from trees proved to be infected by mottle leaf. Transmission tests completed in 1939 proved the wild cherry trees from one isolated locality to be infected by mottle leaf; tests are incomplete for the other group of trees. This may be considered as evidence to support the supposition that mottle leaf is indigenous to wild cherries in the north central part of Washington.

CONTROL

The question of possible control measures is of particular interest to orchardists of the north central part of Washington who have cherry trees affected by mottle leaf. It has already been suggested (*6*) that diseased trees be taken out wherever possible, especially if they are affected to the extent that a profitable crop is not being produced. In discussing control measures, it might be well to point out that a tree once affected by mottle leaf has never been known to recover. Diseased trees cannot be cured by any known treatment, and removal of affected trees to prevent spread to healthy trees is the only suggested method of control. Because diseased trees usually produce good crops for 1 year, and often 2 years, after leaf symptoms are first evident, most growers hesitate to remove them. In most orchards under observation in the north central part of Washington mottle leaf spread very slowly during the years 1934 to 1938, inclusive. In a few orchards it has spread rapidly, however, and the presence of diseased trees is considered a menace to the remaining healthy trees in the orchard. In view of present knowledge of the disease the following suggestions for its control can be made: (1) Remove all trees that have shown leaf symptoms for 2 years or more in orchards where there has been little or no recent spread of mottle leaf; and (2) immediately remove all trees showing symptoms of mottle leaf in orchards where recent spread indicates that such trees are a menace to the remaining healthy trees in the orchard.

In instances where diseased trees have been removed, young trees have been reset in approximately the same location and have remained healthy for the four growing seasons during which they have been under observation.

It has been demonstrated (*3*, *4*) that rootstocks influence the susceptibility of sweet cherry to buckskin, a graft-infectious disease of sweet cherry occurring in California. There is no evidence that the susceptibility of cherries to mottle leaf can be influenced by rootstocks except the observation that naturally infected Bing trees exhibit equally severe leaf symptoms when growing on either mazzard or mahaleb stock.

SUMMARY

Mottle leaf, a virus disease of cherries that has been transmitted artifically only by grafting or by some adaptation of the grafting process, has been observed affecting cherry trees growing in Washington, Oregon, Idaho, California, and British Columbia. It is probable that the disease has been present in the Pacific Northwest for more than 20 years, although it was not proved to be bud- or graft-transmissible until 1935.

The leaves exhibit the most conspicuous symptoms of the disease, although the growth and fruit of the tree are also affected in instances where leaf symptoms are severe.

Considerable variation was found in the leaf symptoms of the different cherry varieties. Under field conditions only four varieties have been found with mottle leaf symptoms. Bing and Napoleon are the two most important commercial varieties that display severe leaf symptoms. Of the 19 cherry varieties inoculated none was immune to mottle leaf, although many were quite tolerant and showed very slight leaf symptoms.

Attempts to transmit the disease to other stone fruits have been mostly unsuccessful because the infective cherry buds did not form a growth union with them. However, under greenhouse conditions where growth union was established between cherry and peach, a preliminary experiment demonstrated that the virus was transmitted through tissues of the peach.

Mottle leaf was transmitted by budding from cherry to cherry, producing definite symptoms in 14 days on trees grown in the greenhouse and in 37 days under field conditions.

Tests demonstrated that mottle leaf could be transmitted by any method where growth union was established between diseased and healthy tissues. No mechanical method of transmission was found to be successful. The manner in which mottle leaf spreads in the field is unknown.

The black cherry aphid (*Myzus cerasi*) was tested as a possible vector of mottle leaf, and no transmission was obtained in these experiments.

Hot-water treatment of bud sticks at 46° C. for 60 minutes and at 49° for 10 minutes failed to inactivate the infective principle under the conditions of the experiment.

Cherry disorders causing leaf malformations that are sometimes confused with mottle leaf are briefly described.

There is no record of a tree having recovered from the disorder once the leaves definitely exhibit mottle leaf symptoms. It is suggested that cherry trees affected by mottle leaf be removed from the orchard to prevent the disease from spreading.

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